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***506084009**

QIAGEN INC

VALENCIA CA

State of West Virginia Department of Administration Quotation **Purchasing Division** 2019 Washington Street East Post Office Box 50130 Charleston, WV 25305-0130

91355-1005

Request for

RFO NUMBER <u>LBS70427</u>

ADDRESS CORRESPONDENCE TO ATTENTION OF:

ROBERTA WAGNER 304-558-0067

800-426-8157

HEALTH AND HUMAN RESOURCES BPH - LABORATORY SERVICES 27220 TURNBERRY LANED

> 167-ELEVENTH AVENUE SOUTH CHARLESTON, WV 25303

304-558-3530 SHIP VIA FOR FREIGHT TERMS TERMS OF SALE DATE PRINTED

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02/11/2007 BID OPENING DATE: 03/13/2007 BID OPENING TIME 01:30PM CAT. QUANTITY ITEM NUMBER UNIT PRICE AMOUNT LINE 0001 EΑ 493-08 BIO-RAD BIO-PLEX 200 SUSPENSION ARRAY 4 qui Chip System - "Superior Equivalent" BIO-RAD BIO-PLEX 200 SUSPENSION ARRAY, CATALOG #171000205, OR EQWAL, TO BE UTILIZED IN THE BIO-TERRORISM LABORATORY AT THE OFFICE OF LABORATORY SERVICES LOCATED AT 167-11TH. AVENUE., SOUTH CHARLESTON WEST VIRGINIA, 25303. THIS EQUIPMENT IS TO BE USED FOR RAPID DETECTION OF POTENTIAL BIOTERRORIST OR OTHER INFECTIOUS AGENTS. SEE ATTACHED SPECIFICATIONS. CANCELLATION: THE DIRECTOR OF PURCHASING RESERVES THE RIGHT TO CANCEL THIS CONTRACT IMMEDIATELY UPON WRITTEN NOTICE TO THE VENDOR IF THE COMMODITIES AND/OR SERVICE SUPPLIED ARE OF AN INFERIOR QUALITY OR DO NOT CONFORM TO THE SPECIFICATIONS OF THE BID AND CONTRACT HEREIN. BANKRUPTCY: IN THE EVENT THE VENDOR/CONTRACTOR FILES FOR BANKRUPTICY PRIOTECTION, THIS CONTRACT IS AUTOMATI-CALLY NULL AND VOID, AND IS TERMINATED WITHOUT FURTHER ORDER. INQUIRIES: WRITTEN QUESTIONS SHALL BE ACCEPTED THROUGH CLOSE OF BUSINESS ON TUESDAY 02/27/2007. QUESTIONS MAY BE SENT VIA USPS, FAX, COURIER, OR EMAIL. IN ORDER TO ASSURE NO VENDOR RECEIVES AN UNFAIR ADVANTAGE, NO SUBSTANTIVE QUESTIONS WILL BE ANSWERED VERBALLY. IF POSSIBLE EMAIL QUESTIONS ARE PREFERRED. ADDRESS INQUIRIES TO: ROBERTA WAGNER DEPARTMENT OF ADMINISTRATION SEE REVERSE SIDE FOR TERMS AND CONDITIONS: ADDRESS CHANGES TO BE NOTED ABOVE TOTA MUNUSEN 95-4141306

GENERAL TERMS & CONDITIONS REQUEST FOR QUOTATION (RFQ) AND REQUEST FOR PROPOSAL (RFP)

- 1. Awards will be made in the best interest of the State of West Virginia.
- 2. The State may accept or reject in part, or in whole, any bid.
- 3. All quotations are governed by the West Virginia Code and the Legislative Rules of the Purchasing Division.
- 4. Prior to any award, the apparent successful vendor must be properly registered with the Purchasing Division and have paid the required \$125.00 registration fee.
- 5. All services performed or goods delivered under State Purchase Orders/Contracts are to be continued for the term of the Purchase Order/Contract, contingent upon funds being appropriated by the Legislature or otherwise being made available. In the event funds are not appropriated or otherwise available for these services or goods, this Purchase Order/Contract becomes void and of no effect after June 30.
- 6. Payment may only be made after the delivery and acceptance of goods or services.
- Interest may be paid for late payment in accordance with the West Virginia Code.
- 8. Vendor preference will be granted upon written request in accordance with the West Virginia Code.
- 9. The State of West Virginia is exempt from federal and state taxes and will not pay or reimburse such taxes.
- 10. The Director of Purchasing may cancel any Purchase Order/Contract upon 30 days written notice to the seller.
- 11. The laws of the State of West Virginia and the *Legislative Rules* of the Purchasing Division shall govern all rights and duties under the Contract, including without limitation the validity of this Purchase Order/Contract.
- 12. Any reference to automatic renewal is hereby deleted. The Contract may be renewed only upon mutual written agreement of the parties.
- 13. BANKRUPTCY: In the event the vendor/contractor files for bankruptcy protection, this contract is automatically null and void, and is terminated without further order.
- 14. HIPAA Business Associate Addendum The West Viginia State Government HIPAA Business Associate Addendum (BAA), approved by the Attorney General, and available online at the Purchasing Division's web site (http://www.state.wv.us/admin/purchase/vrc/hipaa.htm) is hereby made part of the agreement. Provided that, the Agency meets the definition of a Covered Entity (45 CFR §160.103) and will be disclosing Protected Health Information (45 CFR §160.103) to the vendor.

INSTRUCTIONS TO BIDDERS

- 1. Use the quotation forms provided by the Purchasing Division.
- 2. SPECIFICATIONS: Items offered must be in compliance with the specifications. Any deviation from the specifications must be clearly indicated by the bidder. Alternates offered by the bidder as EQUAL to the specifications must be clearly defined. A bidder offering an alternate should attach complete specifications and literature to the bid. The Purchasing Division may waive minor deviations to specifications.
- 3. Complete all sections of the quotation form.
- Unit prices shall prevail in cases of discrepancy.
- 5. All quotations are considered F.O.B. destination unless alternate shipping terms are clearly identified in the quotation.
- 6. BID SUBMISSION: All quotations must be delivered by the bidder to the office listed below prior to the date and time of the bid opening. Failure of the bidder to deliver the quotations on time will result in bid disqualifications.

SIGNED BID TO:

Department of Administration Purchasing Division 2019 Washington Street East Post Office Box 50130 Charleston, WV 25305-0130



State of West Virginia
Department of Administration
Purchasing Division
2019 Washington Street East Post Office Box 50130 Charleston, WV 25305-0130

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ADDRESS CHANGES TO BE NOTED ABOVE

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WHEN RESPONDING TO RFQ, INSERT NAME AND ADDRESS IN SPACE ABOVE LABELED 'VENDOR'



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WHEN RESPONDING TO RFQ, INSERT NAME AND ADDRESS IN SPACE ABOVE LABELED 'VENDOR'



State of West Virginia Department of Administration **Purchasing Division** 2019 Washington Street East Post Office Box 50130 Charleston, WV 25305-0130

Request for Quotation

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ADDRESS CORRESPONDENCE TO ATTENTION OF

ROBERTA WAGNER

304-558-0067

*506084009 800-426-8157 QIAGEN INC 27220 TURNBERRY LANED

VALENCIA CA 91355-1005

HEALTH AND HUMAN RESOURCES BPH - LABORATORY SERVICES

167-ELEVENTH AVENUE SOUTH CHARLESTON, WV 25303 304-558-3530

ADDRESS CHANGES TO BE NOTED ABOVE

DATE PRINTED TERMS OF SALE F.O.B. SHIP VIA FREIGHT TERMS 02/11/2007 **BID OPENING DATE:** 03/13/2007 **BID OPENING TIME** 01:30PM CAT LINE QUANTITY UNIT PRICE ITEM NUMBER **AMOUNT** YEARS IMMEDIATELY PRECEDING SUBMISSION OF THIS BID. BIDDER UNDERSTANDS IF THE SECRETARY OF TAX & REVENUE DETERMINES THAT A BIDDER RECEIVING PREFERENCE HAS FAILED TO CONTINUE TO MEET THE REQUIREMENTS FOR SUCH PREFERENCE, THE SECRETARY MAY ORDER THE DIRECTOR OF PURCHASING TO: (A) RESCIND THE CONTRACT OR PURCHASE ORDER ISSUED; OR (B) ASSESS A PENALTY AGAINST SUCH BIDDER IN AN AMOUNT NOT TO EXCEED 5% OF THE BID AMOUNT AND THAT SUCH PENALTY WILL BE PAID TO THE CONTRACTING AGENCY OR DEDUCTED FROM ANY UNPAID BALANCE ON THE CONTRACT OR PURCHASE ORDER. BY SUBMISSION OF THIS CERTIFICATE, BIDDER AGREES TO DISCLOSE ANY REASONABLY REQUESTED INFORMATION TO THE PURCHASING DIVISION AND AUTHORIZES THE DEPARTMENT OF TAX AND REVENUE TO DISCLOSE TO THE DIRECTOR OF PURCHASING APPROPRIATE INFORMATION VERIFYING THAT BIDDER HAS PAID THE REQUIRED BUSINESS TAXES, PROVIDED THAT SUCH INFORMATION DOES NOT CONTAIN THE AMOUNTS OF TAXES PAID NOR ANY OTHER INFORMATION DEEMED BY THE TAX COMMISSIONER TO BE CONFIDENTIAL. UNDER PENALTY OF LAW FOR FALSE SWEARING (WEST VIRGINIA CODE 61-5-3), BIDDER HEREBY CERTIFIES THAT THIS CERTIFICATE IS TRUE AND ACCURATE IN ALL RESPECTS; AND THAT IF A CONTRACT IS ISSUED TO BIDDER AND IF ANYTHING CONTAINED WITHIN THIS CERTIFICATE CHANGES DURING THE CONTRACT, BIDDER WILL NOTIFY THE PURCHASING TERM OF THE DIVISION IN WRITING IMMEDIATELY. BIDDER: DATE: SEE REVERSE SIDE FOR TERMS AND CONDITIONS 13-622-341

WHEN RESPONDING TO RFQ, INSERT NAME AND ADDRESS IN SPACE ABOVE LABELED 'VENDÓR'

95-4141306



State of West Virginia Department of Administration Quotation Purchasing Division 2019 Washington Street East Post Office Box 50130 Charleston, WV 25305-0130

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WHEN RESPONDING TO RFQ, INSERT NAME AND ADDRESS IN SPACE ABOVE LABELED 'VENDOR'

95-4141306



State of West Virginia
Department of Administration
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Charleston, WV 25305-0130

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SPECIFICATIONS AND REQUIREMENTS

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Equipment Specifications:

- 1. Must be able to analyze a single sample for up to 100 different biomolecules.
- 2. System software must be able to perform calibrations, validation, and monitor the functionality of the system.
- 3. System software must be able to display real-time data as it is generated.
- 4. System must be able to use Bio-Rad Validation and Calibration kits or equivalent to ensure the system is operating properly.
- 5. Assay Reader must have the dimensions of 17x20x9 inches or smaller and weigh 60 pounds or less due to available space in the Bioterrorism Lab.
- 6. Microplate Platform must have the dimensions of 17.3x24x3 inches or smaller and weigh 32 pounds or less due to available space in the Bioterrorism Lab.
- 7. High-Throughput Fluidics System must have the dimensions of 8x12x10 inches or smaller and weigh 20 pounds or less due to available space in the Bioterrorism Lab.
- 8. Assay Reader must have a minimum sample injection rate of 60 microliters per minute.
- 9. Any and all validation and calibrations kits needed for the initial set up must be included in the cost.

Installation & Training Requirements:

- 1. Installation and initial training must be included at no additional cost and must be completed within 30 days of equipment delivery.
- 2. Site training must be performed at the Office of Laboratory Services, 167 11th Avenue, South Charleston, WV 25303.

Warranty Requirements

1. At least one-year equipment warranty to include parts and labor and must be provided at no additional cost.

Shipping and Delivery Requirements

- 1. Shipping terms to be F.O.B. Destination, Prepaid unless vendor states differently when submitting quotation.
- 2. Must be delivered within 60 days of approved purchase order.
- 3. Delivery must be inside delivery.

ATTACHMENT P.O.# LBS70427

This agreement constitutes the entire agreement between the parties, and there are no other terms and conditions applicable to the licenses granted hereunder.

Agreed

INSTRUMENT

Company Name

Signature WINT 3/12/17
Senior Territory Malager
Title

OTAGEN

Agency-Division

AFFIDAVIT

___009

West ∀irginia Code §5A-3-10a states:

No contract or renewal of any contract may be awarded by the state or any of its political subdivisions to any vendor or prospective vendor when the vendor or prospective vendor or a related party to the vendor or prospective vendor is a debtor and the debt owned is an amount greater than one thousand dollars in the aggregate

DEFINITIONS:

"Debt" means any assessment, premium, penalty, fine, tax or other amount of money owed to the state or any of its political subdivisions because of a judgment, fine, permit violation, license assessment, defaulted workers' compensation premium, penalty or other assessment presently delinquent or due and required to be paid to the state or any of its political subdivisions, including any interest or additional penalties accrued thereon.

"Debtor" means any individual, corporation, partnership, association, limited liability company or any other form or business association owing a debt to the state or any of its political subdivisions. "Political subdivision" means any county commission; municipality; county board of education; any instrumentality established by a county or municipality; any separate corporation or instrumentality established by one or more counties or municipalities, as permitted by law; or any public body charged by law with the performance of a government function or whose jurisdiction is coextensive with one or more counties or municipalities. "Related party" means a party, whether an individual, corporation, partnership, association, limited liability company or any other form or business association or other entity whatsoever, related to any vendor by blood, marriage, ownership or contract through which the party has a relationship of ownership or other interest with the vendor so that the party will actually or by effect receive or control a portion of the benefit, profit or other consideration from performance of a vendor contract with the party receiving an amount that meets or exceed five percent of the total contract amount.

EXCEPTION:

The prohibition of this section does not apply where a vendor has contested any tax administered pursuant to chapter eleven of this code, workers' compensation premium, permit fee or environmental fee or assessment and the matter has not become final or where the vendor has entered into a payment plan or agreement and the vendor is not in default of any of the provisions of such plan or agreement.

LICENSING:

Vendors must be licensed and in good standing in accordance with any and all state and local laws and requirements by any state or local agency of West Virginia, including, but not limited to, the West Virginia Secretary of State's Office, the West Virginia Tax Department, West Virginia Insurance Commission, or any other state agencies or political subdivision. Furthermore, the vendor must provide all necessary releases to obtain information to enable the Director or spending unit to verify that the vendor is licensed and in good standing with the above entities.

CONFIDENTIALITY:

The vendor agrees that he or she will not disclose to anyone, directly or indirectly, any such personally identifiable information or other confidential information gained from the agency, unless the individual who is the subject of the information consents to the disclosure in writing or the disclosure is made pursuant to the agency's policies, procedures and rules. Vendors should visit www.state.wv.us/admin/purchase/privacy for the Notice of Agency Confidentiality Policies.

Under penalty of law for false swearing (West Virginia Code, §61-5-3), it is hereby certified that the vendor acknowledges the information in this said affigavit and are in compliance with the requirements as stated.

Vendor's Name: QIAGENPA		
Authorized Signature:	Date:	03/12/07
No Debt Affidavit (Revised 10/13/06)	•	' ' /



March 12th, 2007

State of West Virginia
Department of Administration
Purchasing Department
2019 Washington Street East
Post Office Box 50130
Charleston, WV 25305-0130

In response to RFQ # LBS70427, 03/13/2007 01:30PM,

QIAGEN's LiquiChip system is an assay platform based on xMAP (Luminex) technology that offers the potential to rapidly quantify up to 100 different analytes in a single sample. A wide range of assays for proteomics, drug discovery research, clinical and most recently biodefense/infectious disease agents can be quickly and easily adapted to LiquiChip technology.

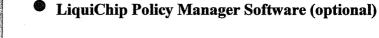
QIAGEN's Agreement MM031207A includes items listed for a grand total of \$59,114. See Agreement attached.

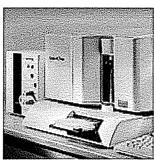
- LiquiChip 200 Reader-A compact bench top analyzer for xMAP assays. Sophisticated laser and optics technologies are combined with a precise fluidics system to deliver high assay sensitivity and robust and reproducible performance. The Reader has a minimum sample injection rate of 60 micro liters per minute (1 micro liter/second). The physical dimensions: 43 cm (17 inches)W x 50.5 cm (20 inches)D x 24.5 cm (9.5 inches) H. Weight: 25 kg (60 lbs)
- LiquiChip Analyzer Software-Latest Luminex Integrated System (IS) software. The IS2.2 software is designed for the clinical and the research laboratory. The IS2.2 offers features for routine measurements—state-of-the art curve fitting algorithms, comprehensive data analysis and viewing tools in a highly intuitive user interface based around assay analysis workflow. There is continuous update/display of the assay progress. (Data analysis, of course will

not run "real-time" but is done once a run is completed using the customer's settings and parameters).

- LiquiChip Microplate Handler- Automated loading and processing of samples from standard 96-well microplates, increasing throughput and allowing walk away operation. A 96-well plate is processed in 35 to 60 minutes, depending on the assay set up and sampling can be started from any given position on the plate. In addition, the LiquiChip Microplate Handler contains a small systems Fluid reservoir that can be used for instrument wash steps. The dimensions are-Height: 8 cm (3 in); Width: 44 cm (17.25 in); Depth: 60 cm (23.5 in); mass: 15kg (33 lb).
- LiquiChip Fluid Module- Processing multiple microplates with no refilling of system-fluid bottles. Also monitors system fluid levels ensuring that the instrument does not "run dry". The dimensions are 24.75 cm (9.75 in) H x 20 cm (8 in)W x 30 cm (11.75 in) D. Weight is 9 kg (20 lb).
- HP computer and TFT flat-screen monitor
- Bar-code scanner-easy sample acquisition
- Calibration and Control Bead Kit-Our kits have been listed on the quote but our LiquiChip is an open system and can be used with all available kits (Bio-Rad's kits can be included) that are specifically designed for Multi Analyte Profiling (xMAP) whether this is protein based or nucleic acid based kit. We have FDA approval in conjunction with specific kits.
- System Fluid with reservoir and waste containers-the liquid/fluidic transporter. The LiquiChip Reader is delivered with both a reservoir and waste container, which holds 1.1 liters each.
- Three-year warranty and yearly Preventative Maintenance agreement includes parts, labor and one annual preventive maintenance (3 visits in total) over a three year period. The LiquiChip hardware carries a 1-year warranty from the date of original purchase.
- Installation & Training- QIAGEN will deliver instrument to the Bioterrorism Laboratory at the Office of Laboratory Services located at 167-11th Avenue., South Charleston, WV 25303 and provide installation and introductory training upon delivery. QIAGEN will install the protocol, test the protocol and backup the system software and protocol. If the Customer delays installation and/or introductory training for more than four weeks after delivery, payment for the instrument is due by Customer.







LiquiChip

Quote



Date March 12th, 2007

QIAGEN Agreement # MM031207A, Response to RFQ#LBS70427

Expires April 30th, 2007

State of West Virginia
Department of Administration
Purchasing Division
2019 Washington Street East
Post Office Box 50130
charleston, WV 25305-0130

QIAGEN Inc.

27220 Tumberry Lane

Valencia, CA 91355

www.qiagen.com

Telephone 661-702-3000

Toll Free 800-426-8157

800-718-2056

\$2,416

Phone: 304-558-0067 Fax: 304-558-4115

e-mail: RWAGNER@WVADMIN.GOV

Contents	Catalogue #	Unit Price	Quantity	Extended Price
<u>LiquiChip Reader -</u> a compact bench top analyzer for xMAP assays. Sophisticated laser and optics technologies are combined with a precise fluidics system to deliver high assay sensitivity and robust and reproducible performance.	9001231	\$58,600	1	\$43,950
<u>LiquiChip Microplate Handler</u> - for automated loading and processing of samples from standard 96 well microplates, increasing throughput and allowing walk away operation.				
<u>LiquiChip Fluid Module</u> for processing of multiple microplates with no refilling of system-fluid bottles and monitors system fluid levels ensuring that the instrument does not "run dry".				
Bar Code Reader				
Computer and Software				
Computer and TFT Monitor - Pentium IV, 933 MHz or higher, at lest 256 MB RAM and 10 GB HD, RW-CD-ROM, MS Windows 2000.				
	LiquiChip Reader - a compact bench top analyzer for xMAP assays. Sophisticated laser and optics technologies are combined with a precise fluidics system to deliver high assay sensitivity and robust and reproducible performance. LiquiChip Microplate Handler - for automated loading and processing of samples from standard 96 well microplates, increasing throughput and allowing walk away operation. LiquiChip Fluid Module - for processing of multiple microplates with no refilling of system-fluid bottles and monitors system fluid levels ensuring that the instrument does not "run dry". Bar Code Reader Computer and Software Computer and TFT Monitor - Pentium IV, 933 MHz or higher, at lest 256 MB RAM and 10 GB HD, RW-CD-ROM, MS	LiquiChip Reader - a compact bench top analyzer for xMAP assays. Sophisticated laser and optics technologies are combined with a precise fluidics system to deliver high assay sensitivity and robust and reproducible performance. LiquiChip Microplate Handler - for automated loading and processing of samples from standard 96 well microplates, increasing throughput and allowing walk away operation. LiquiChip Fluid Module - for processing of multiple microplates with no refilling of system-fluid bottles and monitors system fluid levels ensuring that the instrument does not "run dry". Bar Code Reader Computer and Software Computer and TFT Monitor - Pentium IV, 933 MHz or higher, at lest 256 MB RAM and 10 GB HD, RW-CD-ROM, MS	LiquiChip Reader - a compact bench top analyzer for xMAP 9001231 \$58,600 assays. Sophisticated laser and optics technologies are combined with a precise fluidics system to deliver high assay sensitivity and robust and reproducible performance. LiquiChip Microplate Handler - for automated loading and processing of samples from standard 96 well microplates, increasing throughput and allowing walk away operation. LiquiChip Fluid Module - for processing of multiple microplates with no refilling of system-fluid bottles and monitors system fluid levels ensuring that the instrument does not "run dry". Bar Code Reader Computer and TFT Monitor - Pentium IV, 933 MHz or higher, at lest 256 MB RAM and 10 GB HD, RW-CD-ROM, MS	LiquiChip Reader - a compact bench top analyzer for xMAP 9001231 \$58,600 1 assays. Sophisticated laser and optics technologies are combined with a precise fluidics system to deliver high assay sensitivity and robust and reproducible performance. LiquiChip Microplate Handler - for automated loading and processing of samples from standard 96 well microplates, increasing throughput and allowing walk away operation. LiquiChip Fluid Module - for processing of multiple microplates with no refilling of system-fluid bottles and monitors system fluid levels ensuring that the instrument does not "run dry". Bar Code Reader Computer and Software Computer and TFT Monitor - Pentium IV, 933 MHz or higher, at lest 256 MB RAM and 10 GB HD, RW-CD-ROM, MS

Operating System - IS 2.3 and Developer Workbench software for complete acquisition, analysis, presentation, storage, customer assay development and transfer of assay data.

LiquiChip Analyzer Software <u>LiquiChip Analyzer Software</u>. For the analysis of xMAP protein assay data. The LiquiChip Analyzer software has been developed for fast and efficient processing of multiplex protein assay data generated by any xMAP instrument. Includes one individual software license including security key (USB "dongles").

9016710

\$2,416

<u>Free Software Updates</u> while system is under QIAGEN warranty. Updates are changes to current software versions not new software releases.

	Warranty and Preventive Maintenance			119274	
Extended Warranty	<u>3 Year Warranty -</u> covers the LiquiChip Workstation for 3 years total including all per incident travel and labor charges, all parts and 48-hour priority response.	9236916	\$9,300	1	\$9,300
	3 Year Preventive Maintenance Contract - one guaranteed scheduled visit per year for LiquiChip Workstation preventative maintenance and calibration.				
	Installation and Training				
Installation and Training	<u>Complete System Installation and in Lab System Training -</u> training to take place at time of installation for unlimited number of people.	9237085	\$2,700	1	\$2,700
	Consumables				
System Fluid	5 L System Fluid- (10x concentrate), for 50 L working solution.	922902	\$60	1	\$60
Control Beads	<u>LiquiChip Control Bead Kit -</u> classification control beads (CON1, 5ml): reporter control beads (CON2, 5ml) for 25-30 calibrations.	922912	\$269	1	\$269
Calibration Beads	<u>LiquiChip Calibration Bead Kit -</u> Classification calibration beads (CAL1, 5ml); reporter calibration beads (CAL2, 5ml) for 25-30 Calibrations.	922911	\$269	1	\$269
	Additional items not included with LiquiChip System				
LiquiChip Filter Microplates	<u>LiquiChip Filter Microplates -</u> 96-well filter microplates plus lids, 10 per case.	922920	\$146	1	Optional
QIAvac 96 -	QIAvac 96 - vacuum manifold for processing QIAGEN 96 well filter plates, includes QIAvac 96 top plate, base, waste tray and plate holder.	19504	\$471	1	Optional
	•			Tot	al \$58,964
Quote expires	April 30th, 2007				
Tarme: Nat 30	I Davis		c	Grand	Total \$59.114

Terms: Net 30 Days

Estimated delivery: 2 weeks

Estimated Shipping cost, \$150.00

- 1. All sales are subject to state and local taxes where applicable and are not included in this price quote.
- 2. Shipping charges vary by delivery destination and will be prepaid by QIAGEN and added to the invoice, FOB origin.
- 3. LiquiChip hardware carries a 1-year warranty from the date of original purchase. The computer controller is obtained from an outside vender and carries a 3-year warranty supported by the computer vender. A 3-year warranty can be purchased as described above.

To Place Orders

By Mail:

QIAGEN Inc.

27220 Turnberry Lane Valencia, CA 91355

Customer Service: Shannon Cunningham

By Phone:

1-800-426-8157

Shannon Cunningham ext. 23452

By Fax:

1-800-718-2056

Attention: Shannon Cunningham

<u>Grand Total \$59,114</u>

Marianna Mott

Senior Territory Manager



LiquiChip™ Control Bead Kit

for validation of the classification and reporter channels of the LiquiChip™ Reader

LiquiChip Control Beads are used to validate the bead classification function and the dynamic range of the reporter channel. A mixture of beads with five different color-codes is used to check that the bead classification channel is functioning correctly. A second mixture containing beads with four different concentrations of reporter fluorophore is used to check reporter channel response over the dynamic range of the LiquiChip Reader.

Specifications

CON1 Beads — for validation of the LiquiChip Reader classification channels

Bead makeup:

Five different LiquiChip bead sets with defined target values for Doublet Discriminator (DD),

and classification dye channels (CL 1 and CL 2).

Form:

5 ml stabilized, concentrated bead solution

No. of validations:

5 ml is sufficient for ~25 classification channel validations.

CON2 Beads — for validation of the LiquiChip Reader reporter channel

Bead makeup:

Four different LiquiChip bead sets with defined, lot dependent reporter-fluorescence

intensities.

Form:

5 ml stabilized, concentrated bead solution

No. of validations:

5 ml is sufficient for ~25 reporter channel validations.

Stability and storage: LiquiChip Control Beads CON1 and CON2 are stable for 2 years when stored

at 4°C in the dark. DO NOT FREEZE LiquiChip beads!

Calibration and Control Protocols for your instrument are described in the LiquiChip Workstation User Manual, and the onscreen help function in the software.

Working with LiquiChip beads

All LiquiChip beads and fluorescently labeled detection reagents are light-sensitive and should be protected from light during all stages of storage and use. When pipetting LiquiChip Beads, work quickly to minimize their exposure to light.

Control procedure

- 1. Turn on the instruments, launch the software, and perform the daily startup procedure.
- 2. To validate your instrument, pipet 5 drops of CON1 Beads into well C1 of a 96-well microplate, and 5 drops of CON2 Beads into well D1. Place the plate in the LiquiChip Microplate Handler.
- 3. Click the "CON1" button in the Integrated System software.
- 4. Click the "new con target" button.
- 5. Enter the appropriate lot number, expiration date, and all fifteen target values (see the label in the lid of the LiquiChip Control Bead Kit container) in the appropriate fields.

If the target values are not correctly entered, the control procedure will fail.

- Click "OK" to confirm the target values.
- 7. Click "OK" to start the CON1 validation process.

The instrument validates the classification laser and its detectors.

- Click the "CON2" button in the Integrated System software.
- 9. Click the "new con target" button.
- Enter the appropriate lot number, expiration date, and all four target values (see the label in the lid of the LiquiChip Control Bead Kit container) in the appropriate fields.

If the target values are not correctly entered, the control procedure will fail.

- 11. Click "OK" to confirm target values.
- 12. Click "OK" to start the CON2 validation process.

The instrument validates the reporter laser and its detector.

Ordering Information

Product	Confenis	Cai: No.
LiquiChip Control Beads	Classification Control Beads (CON1, 5 ml);	922912
	Reporter Control Beads (CON2, 5 ml), for 25 validations each	

Patented or patent-pending technology and/or registered or registration-pending trademarks of the QIAGEN Group: QIAGEN®, LiquiChip™.

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

By purchasing this product, which contains fluorescently labeled microsphere products authorized by the Luminex Corporation, you, the customer, aquire the right under Luminex's patent rights to use this product or any portion of the product, including without limitation the microsphere bead products contained herein, only with Luminex's laser based fluorescent analytical test instrumentation.

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GIAGLIV.			L
Australia Tel. 03-9489-3666 Fax 03-9489-3888	Canada Tel. 800-572-9613 Fax 800-713-5951	France Tel. 01-60-920-930 Fax 01-60-920-925	A 0 (0 5
Germany Tel. 02103-29-12400 Fax 02103-29-22000		Japan Tel. 03-5547-0811 Fax 03-5547-0818	N V 2
Switzerland Tel. 061-319-30-31 Fax 061-319-30-33	UK and Ireland Tel. 01293-422-999 Fax 01293-422-922	USA Tel. 800-426-81 <i>57</i> Fax 800-718-20 <i>5</i> 6	5 C T

Distributors:

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LiquiChip™ Calibration Bead Kit

for calibration of the classification and reporter channels of the LiquiChip™ Reader

Calibration of the LiquiChip Reader

LiquiChip Calibration Beads, which have precisely defined classification and reporter fluorescence intensities, are used to normalize the instrument settings for the reporter, doublet discriminator, and both classification channels of the LiquiChip Reader. This is an iterative process, in which the voltage of the laser and the photomultiplier is adjusted exactly to the indicated target value.

The calibration process allows reproducible measurements of fluorescence intensity over time. As fluorescence intensity is temperature-dependent, the LiquiChip Reader should be placed in a constant temperature environment, and a new calibration performed every time the ambient temperature differs by more than 3°C from that of the last calibration. The software that controls the LiquiChip workstation displays an onscreen message if such a calibration is required. Even if the temperature has remained constant we recommend that a calibration be carried out every two weeks.

Calibration and Control Protocols for your instrument are described in the LiquiChip Workstation User Manual, and the onscreen help function in the software.

Specifications

CAL1 Beads — for calibration of the LiquiChip Reader classification channels

Bead makeup:

LiquiChip beads with defined target values for Doublet Discriminator (DD), and classification

dye channels (CL 1 and CL 2).

Form:

5 ml stabilized, concentrated bead solution

No. of validations:

5 ml is sufficient for ~25 classification channel calibrations.

CAL2 Beads — for calibration of the LiquiChip Reader reporter channel

Bead makeup:

A LiquiChip bead set with a defined, lot-dependent reporter-fluorescence intensity.

Form:

5 ml stabilized, concentrated bead solution

No. of validations:

5 ml is sufficient for ~25 reporter channel calibrations.

Stability and

LiquiChip Calibration Beads are stable for 2 years when stored at 4°C in the dark.

storage:

DO NOT FREEZE LiquiChip beads!

Working with LiquiChip beads

All LiquiChip beads and fluorescently labeled detection reagents are light-sensitive and should be protected from light during all stages of storage and use. When pipetting LiquiChip beads, work quickly to minimize their exposure to light.

Calibration procedure

- Turn on the instruments, launch the software and perform the daily startup procedure.
- To calibrate your instrument, pipet 5 drops of CAL1 Beads into well A1 of a 96-well microplate, and 5 drops of CAL2 Beads into well B1. Place the plate in the LiquiChip Microplate Handler.
- Click the "CAL1" button in the Integrated System software.
- Click the "new cal target" button.
- Enter the appropriate lot number, expiration date, DD, CL1 and CL2 target values (see the label in the lid of the LiquiChip Control Bead Kit container) in the appropriate fields.

If the target values are not correctly entered, the instrument will be calibrated against incorrect target values, which may result in misclassification of beads and incorrect reporter signal intensities, making measurements impossible.

- 6. Click "OK" to confirm the target values.
- 7. Click "OK" to start the CAL1 validation process.

The instrument calibrates the classification laser and its detectors.

- 8. Click the "CAL2" button in the Integrated System software.
- Click the "new cal target" button.
- 10. Enter the appropriate lot number, expiration date, and RP1 target value (see the label in the lid of the LiquiChip Control Bead Kit container) in the appropriate fields.

If the target value is not correctly entered, the instrument will be calibrated against an incorrect target value, which may result in incorrect reporter signal intensities, making measurements impossible.

- 11. Click "OK" to confirm the target values.
- 12. Click "OK" to start the CAL2 validation process.

The instrument calibrates the reporter laser and its detector.

Ordering Information

Product	Contents	Cat. No.
LiquiChip Calibration Bead Kit	Classification Calibration Beads (CAL1, 5 ml); Reporter Calibration Beads (CAL2, 5 ml),	922911
	for 25 calibrations each	

Patented or patent-pending technology and/or registered or registration-pending trademarks of the QIAGEN Group: QIAGEN®, LiquiChip™. By purchasing this product, which contains fluorescently labeled microsphere products authorized by the Luminex Corporation, you, the customer, aquire the right under Luminex's patent rights to use this product or any portion of the product, including without limitation the microsphere bead products contained herein, only with Luminex's laser based fluorescent analytical test instrumentation.

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

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QIAGEN:		
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Germany Tel. 02103-29-12400 Fax 02103-29-22000		Japan Tel. 03-5547-0811 Fax 03-5547-0818
	102 12 1 1) te 4

Distributors:

Argentine Tecnolob S.A. (D11) 4555 0010 Austria/Slovenic VWR Internotional GmbH (D1) 576 00 0 Belgium/Luxemburg Westburg b.v. 08001-9815 Brazil Uniscience do Brasil 011 3622 2320 China Gene Company Limited (852)2896-6283 Cyprus Scientinatics Lid (02) 765 416 Czech Republic BIOCCONSUII pod. s.r. o. (270) 2 417 29 792 Desmark VWR International Aps 43 86 87 88 Exptyl Clinido 52 52 72 12 Finland VWR International O. (90) 804 551 Genece BioAnolytics SA, 1(1)-640 03 18 India Genetis, (01)1542 1714 or (01)1515 9346 Stored Westburg (Broei) 140, 08 65508 13/4 or 1-800 20 22 22 0 Keres USS Laboratories, Inc. (20) 928-86 97 Malaysia RESEARCH BIOLARS SDN. BHD. (6003)8070 310 Maxica Guinina Voloner S.A. de CV, (55) 55 25 72 57 The Netherlands Westburg b.v. (033)4950094 New Zeoland Biolob Scientific Idd. (09) 980 6700 or 0800 933 966 Norway VWR International AS 22 90 00 00 Polend Syngen Biotech 59.2.o., (07)13514 10 60 or 06017 06 07 Portugal IZSA PORTUGAL, IDA (21) 422 7312 Singapore Research Biolobs Ph 1dd 273 1066 Slowak Republic BIOC-CONSUII Slovakia spol. s.c.o. (02) 5022 1336 South Africa Southern Cross Biotechnology (Pt) Idd (1921) 67 15166 Spoin RASA, S.A. (93) 902.03.090 Sweden VWR International AS (08) 631 34 00 Taiwan TAIGEN Bioscience Corporation (02) 2880 2913 Thailand Theera Trading Co. Idd. (02) 412-5672 In other countries contact: CIAGEIN, Germany Switzerfond UK and Ireland USA Cross Blotech
Tel. 061-319-30-31 Tel. 01293-422-992 Tel. 800-426-8157 Taiwan TAIGE
Fox 061-319-30-33 Fox 01293-422-922 Fox 800-718-2056 GlaGETR, Ge





LiquiChip™ Carboxy Beads

for covalent immobilization of capture molecules in LiquiChip™ assay procedures

LiquiChip Carboxy Beads have carboxyl (COOH) groups on their surfaces. Through activation of these carboxy groups by N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) and N-hydroxysuccinimide (NHS) all molecules containing a free amino group can be covalently linked to and immobilized on the beads.

Specifications

Surface chemistry:

Carboxyl groups

Binds:

Primary amine groups (after activation using EDC and NHS)

Number of COOH

groups per bead:

 $\sim 1 \times 10^8$

Bead makeup:

Polystyrene, internally dyed with classification fluorophores

Bead size:

 $5.6 \, \mu m$

Form:

Stabilized stock suspension, 2.5 x 10° beads/ml

No. of assays:

1 ml is sufficient for 500 assay points

Volume:

1 ml for 500 data points

Stability and storage: LiquiChip Carboxy Beads are stable for 6 months when stored at 4°C in the dark.

DO NOT FREEZE LiquiChip Beads!

Working with LiquiChip carboxy beads

For detection in LiquiChip assays using Carboxy beads, Streptavidin-R-PE is recommended in combination with a biotin-conjugated secondary antibody.

Beads having other surface chemistries (but different bead codes) can be combined and measured in a single sample. Detailed protocols for coupling capture molecules to LiquiChip Carboxy Beads can be found in the LiquiChip Applications Handbook.

All LiquiChip beads and fluorescently labeled detection reagents are light-sensitive and should be protected from light during all stages of storage and use. When pipetting LiquiChip beads, work quickly to minimize their exposure to light.

Protect the beads from light by wrapping reaction vessels in aluminum foil or by placing reactions in a cupboard, a drawer, or under a box. If the bead-classification fluorophores are exposed to light they become photobleached and can no longer be classified by the instrument, and any attendant reporter fluorescence will not be measured.

Ordering Information

Product	Contents	Cat. No.
LiquiChip Carboxy Beads (500)	Carboxy Beads code 21; 1 ml for 500 assay points	922400
LiquiChip Carboxy Bead Set A (4 x 500)	Carboxy Beads code 11, 13, 15, and 21; 1 ml each for up to 4 x 500 assay points	922402
LiquiChip Carboxy Bead Set B (4 x 500)	Carboxy Beads code 31, 33, 35, and 37; 1 ml each for 4 x 500 assay points	922404
LiquiChip Carboxy Bead Set C (4 x 500)	Carboxy Beads code 40, 42, 44, and 46; 1 ml each for 4 x 500 assay points	922406

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LiquiChip™ System Fluid

for carrying LiquiChip™ assay samples through the LiquiChip Reader

LiquiChip System Fluid carries assay samples through the fluidic paths of the LiquiChip Reader or other xMAP™ based systems. The system fluid contains a defined concentration of salt to ensure that the measured beads flow freely through the measurement cuvette, and are correctly defined and measured.

Specifications

Volume: Cubitainer containing 5 liters 10x concentrate (for 50 liters working solution)

Form: Stabilized concentrated stock solution, with preservative

Stability and storage: Stock solution and 1 in 10 dilutions can be stored for 1 year at 18-25°C.

Do not store LiquiChip System Fluid below 10°C, as salts will precipitate out of solution.

Working with LiquiChip System Fluid

LiquiChip System Fluid is specifically formulated for the fluidic paths of the LiquiChip System and therefore its makeup cannot be altered. Do not add reagents to LiquiChip System Fluid.

Dilute LiquiChip System Fluid 1 in 10 before use (i.e., for 5 liters working solution, add 4500 ml filtered, deionized water to 500 ml system fluid 10x concentrate and stir).

To prevent salt crystallization in the tubing of the LiquiChip Reader, we strongly recommend that you perform a "Soak" operation as part of the LiquiChip workstation daily shutdown procedure. During this procedure all tubing in the LiquiChip Reader is washed with distilled water. Rinse the system fluid reservoir twice with distilled water, fill it with distilled water, and perform a "Soak" operation by clicking the "Soak" button.

Ordering Information

Product	Contents	Cat. No.
LiquiChip System Fluid	LiquiChip System Fluid (10x concentrate, 5 liters),	922902
	for 50 liters working solution	

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LiquiChip[™] beads

For immobilization of capture molecules in LiquiChip and other xMAP™ assays

LiquiChip™ beads and detection reagents enable fast, convenient, and sensitive multiplex protein assays using the LiquiChip workstation. They are highly flexible tools that simplify and accelerate assay development in the fields of drug discovery and development and proteomics.

Efficient, Directed Immobilization of 6xHis-tagged Proteins

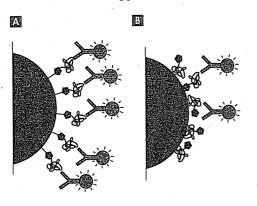


Figure 1 The directed immobilization of óxHis-tagged proteins offered by LiquiChip Ni-NTA and Penta-His Beads improves accessibility of bound capture molecules to reaction partners **E**, compared with randomly immobilized proteins **E**.

Titration of Antibodies into a Multiplex ELISA

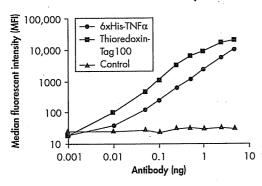


Figure 2 Three LiquiChip Ni-NTA Bead sets were coupled with 6xHis-tagged TNFα; thioredoxin carrying a Tag·100 epitope at its C-terminus; and 6xHis-tagged thioredoxin (control) respectively. The indicated amounts of biotinylated anti-TNFα and Tag·100 Antibodies were added and detected using Streptavidin-R-PE.

LiquiChip Ni-NTA and Penta-His™ Beads

Combining the convenience and speed of xMAP technology with the power of the QIAexpress® System allows one chemistry to be used for the handling of 6xHis-tagged proteins at all stages of the purification and assay process. This increases standardization, simplifies assay development, and minimizes the need for optimization trials. LiquiChip Beads coated with Ni-NTA moieties or Penta-His™ anti-His tag antibodies enable the directed immobilization of biologically active 6xHis-tagged biomolecules; enhancing assay sensitivity, minimizing nonspecific binding, and increasing reproducibility and signal-to-noise ratios (Figure 1). An important advantage offered by the high binding capacity of LiquiChip Ni-NTA Beads is that low affinity interactions can be easily studied.

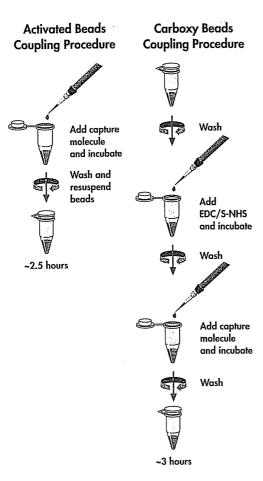
LiquiChip Ni-NTA and Penta-His Beads deliver:

- Universal, directed immobilization of functionally active 6xHis-tagged proteins via their N- or C-terminal 6xHis tag
- Minimal requirement for optimization of coupling conditions
- Tight binding, enabling multiplex assays using different 6xHis-tagged proteins (Figure 2)

Table 1. Binding and coupling properties of LiquiChip beads

	Ni-NTA Beads	Penta-His Beads	Activated Beads	Avidin Beads	Carboxy Beads
Binds	6xHis- tagged proteins	6xHis- tagged proteins	Biomolecules with a free thiol group	Biotinylated biomalecules	Biomolecules with a free amine group
Direct coupling	~	V	V	Х	Х





The mouse monoclonal Penta-His Antibodies coated on the surface of Penta-His beads recognize and bind with high affinity and specificity to an epitope consisting of five consecutive histidine residues, regardless of the amino acids surrounding the motif. As this interaction is an antibody–antigen interaction, it is resistant to most chemical or salt concentration effects that may interfere with the Ni-NTA— 6xHis-tag interaction, such as the presence of imidazole, serum, or hemoglobin.

LiquiChip Activated Beads

LiquiChip Activated Beads offer a fast and convenient "chemistry-free" method for the covalent immobilization of thiol-containing capture molecules, especially antibodies, on LiquiChip beads. The simple coupling procedure does not require tedious pre-activation, multiple wash steps, or chemical reactions (e.g., EDC/S-NHS). Capture molecules are simply incubated with beads in a one-tube procedure and are ready for use after a single, short wash step (see flowchart).

LiquiChip Activated Beads provide:

- Efficient "chemistry-free" immobilization of biomolecules containing a thiol group (e.g., antibodies, cysteine-containing peptides, and GSTfusion proteins)
- A one-tube procedure with no need for pre-activation or multiple wash steps

Protease Assay Using the LiquiChip System

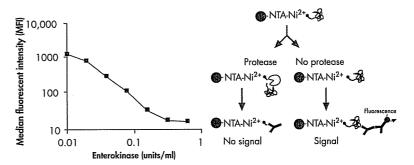


Figure 3 Protease assay using LiquiChip Ni-NTA Beads. Thioredoxin with a 6xHis tag at its N-terminus and a Tag-100 epitope at its C-terminus was coupled to LiquiChip Ni-NTA Beads. The beads were divided into equal aliquots, and different amounts of enterokinase were added to each aliquot. The reactions were incubated for 3 h at 37°C. Residual uncleaved thioredoxin was measured using a monoclonal Alexa Fluor® 532-labeled Tag-100 Antibody.

Sandwich Assay Using LiquiChip Activated and Carboxy Beads

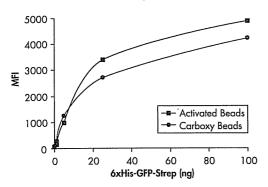


Figure 4 Sandwich antibody assay using antibody-coated Activated and Carboxy beads. For each bead type, 2.5 x 10st LiquiChip beads were coated with 10 µg Streptag Antibody and incubated with increasing amounts (0–100 ng) of green fluorescent protein (GFP) with a 6xHis tag at the N-terminus and a Streptag at the C-terminus. Detection was performed using Penta-His Biotin Conjugate and Streptavidin–R-PE.

Kinase Assay using LiquiChip Activated Beads

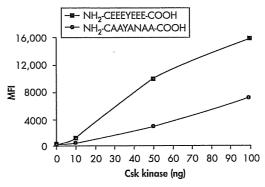


Figure 5 Substrate peptides (10 μg each) were coupled via an N-terminal cysteine residue to 2.5 x 10^s LiquiChip Activated Beads. The peptide-coated LiquiChip beads were incubated for 1 h at 30°C with the indicated amounts of purified Csk kinase. Phosphorylation of the peptide was detected by sequentially adding a phosphotyrosine-specific mouse monoclonal antibody, a goat anti-mouse biotin conjugate, and Streptavidin-PE.

LiquiChip Avidin Beads

LiquiChip Avidin Beads are designed for the immobilization of biotinylated biomolecules, such as antibodies or nucleic acids, using the high-affinity avidin-biotin interaction. The beads are covered with a proprietary, optimized form of avidin that binds biotin with high affinity and specificity, and delivers low nonspecific binding.

LiquiChip Avidin Beads provide:

- Efficient immobilization of biotinylated biomolecules
- Low nonspecific binding

LiquiChip Carboxy Beads

LiquiChip Carboxy Beads are designed for general-purpose use and allow covalent coupling of any biomolecule that carries amino groups on its surface. Biomolecules are bound via carboxy groups on the bead's surface using proven carbodiimide coupling chemistry.

LiquiChip Carboxy Beads provide:

- ₩ Versatile, general-purpose immobilization of virtually any biomolecule
- Proven carbodiimide coupling chemistry

QIAGEN also offers a range of LiquiChip detection reagents and accessories for highly sensitive, specific, and flexible detection of analytes. Visit www.qiagen.com/liquichip for more details.

Ordering Information

Product	Contents	Cat. no.	Price (US\$)
LiquiChip Ni-NTA and Penta-His Bea	ds — for immobilization of 6xHis-tagged proteins		
LiquiChip Ni-NTA Beads (500)	Ni-NTA Beads code 52; 0.25 ml for 500 homogeneous assay points	922500	Inquire
LiquiChip Ni-NTA Bead Set A (4 x 500)*	Ni-NTA Beads code 50, 52, 54, and 58; 0.25 ml each for 4 x 500 homogeneous assay points	922501	Inquire
LiquiChip Penta·His Beads (500)	Penta·His Beads code 81; 0.25 ml for 500 homogeneous assay points	922520	Inquire
LiquiChip Penta·His Bead Set A (4 x 500)*	Penta·His Beads code 75, 77, 79, and 81; 0.25 ml each for 4×500 homogeneous assay points	922521	Inquire
LiquiChip Activated and Carboxy Be	ads — for immobilization of nontagged biomolecules		
LiquiChip Activated Beads (500)	Activated Beads code 61; 0.5 ml for 500 homogeneous assay points	922540	Inquire
LiquiChip Activated Bead Set A (4 x 500)*	Activated Beads code 61, 62, 63, and 64; 0.5 ml each for 4×500 homogeneous assay points	922541	Inquire
LiquiChip Carboxy Beads (500)	Carboxy Beads code 21; 1 ml for 500 assay points	922400	Inquire
LiquiChip Carboxy Bead Set A (4 x 500)*	Carboxy Beads code 11, 13, 15, and 21; 1 ml each for 4 x 500 assay points	922402	Inquire
LiquiChip Avidin Beads — for immo	bilization of biotinylated biomolecules		
LiquiChip Avidin Beads (500)	Avidin Beads code 48; 0.25 ml for 500 homogeneous assay points	922420	Inquire
LiquiChip Avidin Bead Set A (4 x 500)*	Avidin Beads code 41, 43, 45, and 48; 0.25 ml each for 4×500 homogeneous assay points	922421	Inquire

 $^{^{\}star}$ Additional bead sets with different bead codes are available; please inquire.

Visit <u>www.qiagen.com/liquichip</u> to learn more about the LiquiChip System — the complete solution for protein-based suspension arrays!

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QIAGEN Reference Database



WSX-1 is required for resistance to Trypanosoma cruzi infection by regulation of proinflammatory cytokine production

Hamano S, Himeno K, Miyazaki Y, Ishii K, Yamanaka A, Takeda A, Zhang M, Hisaeda H, Mak TW, Yoshimura A, Yoshida H *Immunity* 2003 Nov;5: 657-67

use of LiquiChip device and LiquiChip Mouse Cytokine Kit

WSX-1 is a class I cytokine receptor with homology to the IL-12 receptors and is essential for resistance to Leishmania major infection. In the present study, we demonstrated that WSX-1 was also required for resistance to Trypanosoma cruzi. WSX-1-/- mice exhibited prolonged parasitemia, severe liver injury, and increased mortality over wild-type mice. WSX-1-/- splenocytes produced enhanced levels of Th2 cytokines, which were responsible for the prolonged parasitemia. Massive necroinflammatory lesions were observed in the liver of infected WSX-1-/- mice, and IFN-gamma that was overproduced in WSX-1-/- mice compared with wild-type mice was responsible for the lesions. In addition, vast amounts of various proinflammatory cytokines, including IL-6 and TNF-alpha, were produced by liver mononuclear cells in WSX-1-/- mice. Thus, during T. cruzi infection, WSX-1 suppresses liver injury by regulating production of proinflammatory cytokines, while controlling parasitemia by suppression of Th2 responses, demonstrating its novel role as an inhibitory regulator of cytokine production.

Multiplex assay for detection of strain-specific antibodies against the two variable regions of the G protein of respiratory syncytial virus

Jones LP, Zheng HQ, Karron RA, Peret TC, Tsou C, Anderson LJ

Clin. Diagn. Lab. Immunol. 2002 May;3: 633-8

The role of strain differences in respiratory syncytial virus (RSV) disease has not been clearly defined. To investigate the possibility that strain differences contribute to susceptibility to repeat infections, we developed assays to detect antibodies to the two variable regions of the RSV G protein by cloning and expressing the internal variable region at amino acids (aa) 60 to 172 (g1) and the carboxy-terminal variable region at aa 193 to the carboxy terminus (g2) from different genotypes of RSV. The purified proteins were covalently linked to beads with different proportions of red and orange fluorescent dyes and reacted against serum specimens. Antibody reacting against the differently colored beads, and thus against different G polypeptides, was detected by use of flow cytometry and the Luminex system. This assay system detected group- and, to some extent, genotype-specific responses to RSV infection and can be used to investigate the role of strain differences in RSV disease.

Quantitation of DNA sequences in environmental PCR products by a multiplexed, bead-based method

Spiro A, Lowe M

Appl. Environ. Microbiol. 2002 Feb; 2: 1010-3

A first application of a multiplexed, bead-based method is described for determining the abundances of target sequences in an environmental PCR product. Target sequences as little as 0.3% of the total amount of DNA can be quantified. Tests were conducted on 16S ribosomal DNA sequences from microorganisms collected from contaminated groundwater.

Modulation of bacterial growth by tumor necrosis factor-alpha in vitro and in vivo

Lee JH, Del Sorbo L, Khine AA, de Azavedo J, Low DE, Bell D, Uhlig S, Slutsky AS, Zhang H

Am. J. Respir. Crit. Care Med. 2003 Dec;12: 1462-70

use of LiquiChip mouse Cytokine Kit and LiquiChip Device, samples: lung lavage fluid

Tumor necrosis factor-alpha (TNF-alpha) plays an important role in innate immunity. Recent in vitro studies have shown that TNF-alpha may also serve as a growth factor for some bacteria. We examined the physiologic relevance of this phenomenon both in vitro and in vivo. Recombinant mouse TNF-alpha increased in vitro proliferation of Escherichia coli but not Pseudomonas aeruginosa in a concentration-dependent manner, and this effect was attenuated by anti-TNF-alpha antibodies. However, in vivo, TNF-alpha gene-deficient (TNF-alpha-/-) mice showed higher mortality than wild-type (TNF-alpha+/+) mice after inoculation of intranasal bacteria. An impaired bacterial clearance in TNF-alpha-/- mice was associated with decreased systemic concentrations of chemokine macrophage inflammatory protein-2, reduced pulmonary neutrophil recruitment, and depressed expression of neutrophil CD11b and CD16/CD32, suggesting that the effect of TNF-alpha on E. coli growth was outweighed by the

recruited neutrophils. We also demonstrated that neutropenic TNF-alpha+/+ mice had approximately 100-fold higher E. coli counts in their lungs than TNF-alpha-/- mice, although survival rates in both groups were similar. We conclude that TNF-alpha augments E. coli growth in vitro and in vivo. However, in vivo, this effect becomes only apparent in neutropenic animals. The relevance of these findings for immune compromised patients remains to be investigated.

Development of a microsphere-based serologic multiplexed fluorescent immunoassay and a reverse transcriptase PCR assay to detect murine norovirus 1 infection in mice

Hsu CC, Wobus CE, Steffen EK, Riley LK, Livingston RS

Clin. Diagn. Lab. Immunol. 2005 Oct;10: 1145-51

Murine norovirus 1 (MNV-1) is a newly recognized pathogen of mice that causes lethal infection in mice deficient in components of the innate immune response but not in wild-type 129 mice. In this study, in vitro-propagated MNV-1 was used as antigen to develop a multiplexed fluorescent immunoassay (MFI) to detect antibodies to MNV-1 in infected mice. The MNV-1 MFI was 100% specific and 100% sensitive in detecting anti-MNV-1 antibody in sera from experimentally infected mice. Testing of a large number of mouse serum samples (n = 12,639) submitted from contemporary laboratory mouse colonies in the United States and Canada revealed that 22.1% of these sera contained antibodies to MNV-1, indicating infection with MNV-1 is widespread in research mice. In addition, a reverse transcriptase PCR primer pair with a sensitivity of 25 virus copies was developed and used to demonstrate that MNV-1 RNA could be detected in the spleen, mesenteric lymph node, and jejunum from some experimentally infected mice 5 weeks postinoculation. These diagnostic assays provide the necessary tools to define the MNV-1 infection status of research mice and to aid in the establishment of laboratory mouse colonies free of MNV-1 infection.

Intercellular adhesion molecule-1 mediates cellular cross-talk between parenchymal and immune cells after lipopolysaccharide neutralization

Lee JH, Del Sorbo L, Uhlig S, Porro GA, Whitehead T, Voglis S, Liu M, Slutsky AS, Zhang H

J. Immunol. 2004 Jan;1: 608-16

use of LiquiChip human and mouse Cytokine Kit samples: primary lung explants from rat and mouse, human lung epithelial A549 cells

The mechanisms by which parenchymal cells interact with immune cells, particularly after removal of LPS, remain unknown. Lung explants from rats, mice deficient in the TNF gene, or human lung epithelial A549 cells were treated with LPS and washed, before naive alveolar macrophages, bone marrow monocytes, or PBMC, respectively, were added to the cultures. When the immune cells were cocultured with LPS-challenged explants or A549 cells, TNF production was greatly enhanced. This was not affected by neutralization of LPS with polymyxin B. The LPS-induced increase in the expression of ICAM-1 on A549 cells correlated with TNF production by PBMC. The cellular cross talk leading to the TNF response was blunted by an anti-ICAM-1 Ab and an ICAM-1 antisense oligonucleotide. In A549 cells, a persistent decrease in the concentration of intracellular cAMP was associated with colocalization of LPS into Toll-like receptor 4 and the Golgi apparatus, resulting in increased ICAM-1 expression. Inhibition of LPS internalization by cytochalasin D or treatment with dibutyryl cAMP attenuated ICAM-1 expression and TNF production by PBMC. In conclusion, lung epithelial cells are not bystanders, but possess memory of LPS through the expression of ICAM-1 that interacts with and activates leukocytes. This may provide an explanation for the failure of anti-LPS therapies in sepsis trials.

Hyperproduction of proinflammatory cytokines by WSX-1-deficient NKT cells in concanavalin A-induced hepatitis

Yamanaka A, Hamano S, Miyazaki Y, Ishii K, Takeda A, Mak TW, Himeno K, Yoshimura A, Yoshida H

J. Immunol. 2004 Mar; 6: 3590-6

use of LiquiChip Mouse 10 Cytokine Kit on Luminex 100 device, samples: sera

Administration of Con A induces liver injury that is considered to be an experimental model for human autoimmune or viral hepatitis, where immunopathology plays roles mediated by activated lymphocytes, especially NK1.1+ CD3+ NKT cells, and inflammatory cytokines, including IFN-gamma and IL-4. In the present study we investigated the role of WSX-1, a component of IL-27R, in Con A-induced hepatitis by taking advantage of WSX-1 knockout mice. WSX-1-deficient mice were more susceptible to Con A treatment than wild-type mice, showing serum alanine aminotransferase elevation and massive necrosis in the liver. Although the development of NKT cells appeared normal in WSX-1 knockout mice, purified NKT cells from the knockout mice produced more IFN-gamma and IL-4 than those from wild-type mice in response to stimulation with Con A both in vitro and in vivo. In addition, hyperproduction of proinflammatory cytokines, including IL-1, IL-6, and TNF-alpha, was observed in the

knockout mice after Con A administration. These data revealed a novel role for WSX-1 as an inhibitory regulator of cytokine production and inflammation in Con A-induced hepatitis.