EpiQuant™ EGFR Pathway Magnetic Bead Panel

96 Well Plate Assay

Cat. # MPEQMAG-110K, MPEQMG-110K-PX22

MILLIPLEX® MAP

EpiQuant™ EGFR PATHWAY MAGNETIC BEAD PANEL
96 Well Plate Assay

MPEQMAG-110K
MPEQMG-110K-PX22 (premixed)

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES

INTRODUCTION

MILLIPLEX® *MAP* EpiQuant™ technology uses computational algorithms to identify unique, continuous linear sequences (EpiQuant Sequences) in proteins. EpiQuant antibodies generated against EpiQuant sequences have pre-defined target specificity. Protein abundance and/or protein phosphorylation measurements are made at the peptide level, using EpiQuant antibodies, following proteolytic fragmentation of samples and liberation of EpiQuant-bearing peptides.

A unique feature of the EpiQuant sample processing protocol is fragmentation or digestion of the protein sample. This process has a number of benefits:

- Protein:protein interactions are disrupted.
- A synthetic peptide can be used as a quantitative standard.
- Specificity of the antibodies can be narrowed to a small linear peptide sequence.
- Cross-reactivity with similar peptide sequences in other proteins can be predicted and avoided.
- Residual enzymatic activity of the sample (e.g. phosphatases and proteases) is quenched.

EpiQuant technology represents a major advance in the design and implementation of quantitative immunoassays.

The EGFR Pathway Panel is used for the assay of cellular lysates prepared utilizing the EpiQuant Sample Preparation Kit (MPEQ-SP, purchased separately). **ONLY SAMPLES PREPARED USING THE MPEQ-SP KIT WILL GENERATE RELIABLE RESULTS IN THIS ASSAY**.

The MILLIPLEX *MAP* EpiQuant EGFR Pathway Panel enables the measurement of target specific phosphorylation events and total EGFR. Coupled with the Luminex xMAP® platform in a **magnetic bead** format, you receive the advantage of ideal speed and sensitivity, allowing quantitative multiplex detection of multiple analytes simultaneously, which can dramatically improve productivity.

Millipore's MILLIPLEX *MAP* EpiQuant EGFR Pathway Panel is the most versatile system available for Cell Signaling research.

- MILLIPLEX *MAP* offers you the ability to:
    - Select a 22-plex pre-mixed kit or
    - Choose any combination of analytes from our panel of 22 analytes to design a custom kit that better meets your needs
- A convenient "all-in-one" box format gives you the assurance that you will have all the necessary reagents you need to run your assay.

Millipore's MILLIPLEX *MAP* EpiQuant EGFR Pathway Panel is to be used for the simultaneous quantification of the following 22 phosphorylated proteins: EGFR (2 sites), Csk, Shc, FAK (2 sites), ErbB3 (HER3), PLCγ1, PLCγ2, HER2, PI3Kp110δ, STAT3, Gab1, Gab2 (3 sites), FRS2, ERK1/2, PKCμ, SPRY2, and total EGFR and TAFII68 (loading control).

This kit is for research purposes only.

Please read entire protocol before use.

It is important to use same assay incubation conditions throughout your study.

PRINCIPLE

MILLIPLEX MAP is based on the Luminex® xMAP® technology — one of the fastest growing and most respected multiplex technologies offering applications throughout the life-sciences, and is capable of performing a variety of bioassays including immunoassays on the surface of fluorescent-coded magnetic beads known as MagPlex ™-C microspheres.

- Luminex uses proprietary techniques to internally color-code microspheres with two fluorescent dyes. Through precise concentrations of two dyes, 100 distinctly colored bead sets can be created, each of which is coated with a specific capture antibody.
- After an analyte from a test sample is captured by the bead, a biotinylated detection antibody is introduced.
- The reaction mixture is then incubated with Streptavidin PE conjugate, the reporter molecule, to complete the reaction on the surface of each microsphere.
- The microspheres are allowed to pass rapidly through a laser, which excites the internal dyes marking the microsphere set. A second laser excites PE, the fluorescent dye on the reporter molecule.
- Finally, high-speed digital-signal processors identify each individual microsphere and quantify the result of its bioassay, based on fluorescent reporter signals.

The capability of adding multiple conjugated beads to each sample results in the ability to obtain multiple results from each sample. Open-architecture xMAP technology enables multiplexing of many types of bioassays reducing time, labor and costs over traditional methods.

STORAGE CONDITIONS UPON RECEIPT

- Recommended storage for kit components is 2 - 8°C.
- Once the standards and controls have been reconstituted, immediately transfer contents into polypropylene vials. **DO NOT STORE RECONSITUTED STANDARDS OR CONTROLS IN GLASS VIALS**. For long-term storage, freeze reconstituted standards and controls at ≤ -20°C. Avoid multiple (>2) freeze thaw cycles.
- **DO NOT FREEZE Antibody-Immobilized Beads, Detection Antibody, and Streptavidin-Phycoerythrin**.

REAGENTS SUPPLIED

Note: Store all reagents at 2 – 8 °C

REAGENTS SUPPLIED	CATALOG NUMBER	VOLUME	QUANTITY
EpiQuant EGFR Panel Standard 1	MPEQ-8110-1	lyophilized	1 vial
EpiQuant EGFR Panel Standard 2	MPEQ-8110-2	lyophilized	1 vial
EpiQuant EGFR Panel Quality Controls 1 and 2	MPEQ-6110	lyophilized	2 vials
Set of one 96-Well Plate with 2 Sealers		----------	1 plate 2 sealers
EpiQuant Assay Buffer (10X)	MPEQ-AB	50 mL	1 bottle
EpiQuant Dilution Buffer	MPEQ-DB	3 mL	1 bottle
EpiQuant EGFR Detection Antibody (25X)	MPEQ-1110	150 µL	1 tube
SAPE (25X)	45-001D	115 µL	1 tube
Mixing Bottle (not provided with premixed panel)		----------	2 bottles

MILLIPLEX *MAP* EpiQuant EGFR Pathway Premixed Magnetic Beads:

Premixed 22-plex Beads (1X)	EQ110MAG-PMX22	3.2 mL	1 bottle

Human Cytokine / Chemokine Antibody-Immobilized Magnetic Beads:

Bead/Analyte Name	Luminex Magnetic Bead Region	Customizable 22 Analytes (50X concentration, 90µL) Available	Cat. #	22-Plex Premixed Beads
Csk (pY304)	13	✓	EQCSK-Y304MG	✓
EGFR (pY1110/1125)	15	✓	EQEGFR-Y1110MG	✓
EGFR (pY1069/1092)	78	✓	EQEGFR-Y1069MG	✓
EGFR total	19	✓	EQEGFR-T0TALMG	✓
ErbB3 (pY1197/1199/1262/1276/1289/1307)	25	✓	EQERBB3-Y1197MG	✓
ERK1/2 (pY204/187)	27	✓	EQERK1/2-Y204MG	✓
FAK (pY397/407)	29	✓	EQFAK-Y397MG	✓
FAK (pY861)	33	✓	EQFAK-Y861MG	✓
FRS2 (pY436)	35	✓	EQFRS2-Y436MG	✓
Gab1 (pY285/307/317)	37	✓	EQGAB1-Y285MG	✓
Gab2 (pY266)	39	✓	EQGAB2-Y266MG	✓
Gab2 (pY584)	43	✓	EQGAB2-Y584MG	✓
Gab2 (pY614)	45	✓	EQGAB2-Y614MG	✓
HER2 (pY1023)	47	✓	EQHER2-Y1023MG	✓
PKCµ (pY502)	57	✓	EQPKCM-Y502MG	✓
Pl3Kp110δ (pY485)	61	✓	EQPI3K-Y485MG	✓
PLCγ1 (pY1253)	63	✓	EQPLCG1-Y1253MG	✓
PLCγ2 (pY1197/1217)	65	✓	EQPLCG2-Y1197MG	✓
Shc (pY349/350)	67	✓	EQSHC-Y349MG	✓
SPRY2 (pY55)	73	✓	EQSPRY2-Y55MG	✓
STAT3 (pY705)	75	✓	EQSTAT3-Y705MG	✓
TAFII68 (loading control)	77	✓	EQLC1-MG	✓

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents

1. Luminex Sheath Fluid (Luminex Catalogue #40-50000) or Luminex Drive Fluid (Luminex Catlogue # MPXDF-4PK)

Instrumentation / Materials

1. Adjustable Pipettes with Tips capable of delivering 25 µL to 1000 µl
2. Multichannel Pipettes capable of delivering 5 µl to 50 µl or 25 µl to 200 µl
3. Reagent Reservoirs
4. Polypropylene Microfuge Tubes
5. Rubber Bands
6. Aluminum Foil
7. Absorbent Pads
8. Laboratory Vortex Mixer
9. Sonicator (Branson Ultrasonic Cleaner Model # B200 or equivalent)
10. Titer Plate Shaker (Lab-Line Instruments Model #4625 or equivalent)
11. Luminex 200™, HTS, FLEXMAP 3D™ or MAGPIX® with xPONENT software by Luminex Corporation
12. Automatic Plate washer for magnetic beads (Bio-Tek ELx405, Millipore catalog #40-015 or equivalent) or Hand held Magnetic Separation Block (Millipore catalog # 40-285 or equivalent)

Note: If a plate washer or hand held magnetic separation block for magnetic beads is not available, one can use a microtiter filter plate (Millipore Catalog #MX-PLATE) to run the assay using a Vacuum Filtration Unit (Millipore Vacuum Manifold Catalog #MSVMHTS00 or equivalent with Millipore Vacuum Pump Catalog #WP6111560 or equivalent).

SAFETY PRECAUTIONS

- All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.
- Sodium Azide or Proclin has been added to some reagents as a preservative. Although the concentrations are low, Sodium Azide and Proclin may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build up.

TECHNICAL GUIDELINES

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. The following notes should be reviewed and understood before the assay is set-up.

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use beyond the expiration date on the label.
- Do not mix or substitute reagents with those from other lots or sources.

- The Antibody-Immobilized Beads are light sensitive and must be protected from light at all times. Cover the assay plate containing beads with opaque plate lid or aluminum foil during all incubation steps.
- It is important to allow all reagents to warm to room temperature (20-25°C) before use in the assay.
- Incomplete washing can adversely affect the assay outcome. All washing must be performed with the Assay Buffer provided.
- After hydration, all standards and controls must be transferred to polypropylene tubes.
- The standards prepared by serial dilution must be used within 1 hour of preparation. Discard any unused standards except the standard stock which may be stored at ≤ -20°C for 1 month and at ≤ -80°C for greater than one month.
- Any unused mixed Antibody-Immobilized Beads may be stored in the Bead Mix bottle at 2-8°C for up to one month.
- During the preparation of the standard curve, make certain to mix the higher concentration well before making the next dilution. Use a new tip with each dilution.
- The plate should be read immediately after the assay is finished. If, however, the plate cannot be read immediately, seal the plate, cover with aluminum foil or an opaque lid, and store the plate at 2-8°C for up to 24 hours. Prior to reading, agitate the plate on the plate shaker at room temperature for 10 minutes. Delay in reading a plate may result in decreased sensitivity for some analytes.
- The titer plate shaker should be set at a speed to provide maximum orbital mixing without splashing of liquid outside the wells. For the recommended plate shaker, this would be a setting of 5-7, which is approximately 500-800 rpm.
- Ensure that the needle probe is clean. This may be achieved by sonication and/or alcohol flushes.
- When reading the assay on Luminex 200™, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 4 alignment discs. When reading the assay on FLEXMAP 3D™, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 1 alignment disc. When reading the assay on MAGPIX, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 2 alignment disc.
- Vortex all reagents well before adding to plate.

PREPARATION OF REAGENTS FOR IMMUNOASSAY

A. Preparation of Assay Buffer

Bring Assay Buffer to room temperature and mix to bring all salts into solution. Combine bottle contents of EpiQuant Assay Buffer (10X) with 450 mL ultrapure water. Mix well.

B. Preparation of Antibody-Immobilized Beads

1. If premixed beads are used, sonicate the premixed bead bottle 30 seconds and then vortex for 1 minute before use, no further preparation is needed.

2, For individual vials of beads, sonicate each bead vial for 30 seconds and vortex for 1 minute. Add 60 µL from each bead vial to the Mixing Bottle and bring final volume to 3.0 mL with 1X Assay Buffer. Vortex the mixed beads well. Unused prepared beads may be stored at 2-8°C for up to one month. (Note: Due to the composition of magnetic beads, you may notice a slight color in the bead solution. This does not affect the performance of the beads or the kit.)

Example 1: When using 5 EpiQuant beads, add 60 µL from each of the 5 bead sets to the Mixing Bottle. Then add 2.7 mL 1X Assay Buffer.

Example 2: When using 15 EpiQuant beads, add 60 µL from each of the 15 bead sets to the Mixing Bottle. Then add 2.1 mL 1X Assay Buffer.

C. Preparation of EpiQuant Quality Controls

Reconstitute Quality Control 1 and Quality Control 2 by adding 250 µL Dilution Buffer to each. Invert the vial several times to mix. Vortex the vial for 10 seconds. Allow the vial to sit for 5-10 minutes and then transfer the controls to appropriately labeled polypropylene microfuge tubes. Any unused portion may be stored at ≤ -20°C.

D. Preparation of EpiQuant Peptide Standard

1. Reconstitute the EpiQuant Peptide Standard with Dilution Buffer according to the following chart.

Kit Used	Standard Used	Volume MPEQ-DB used
MPEQMG-110K-PX22	MPEQ-8110-1	250 µL
	MPEQ-8110-2	250 µL
MPEQMAG-110K with EQLC1-MG	MPEQ-8110-1	250 µL
	MPEQ-8110-2	250 µL
MPEQMAG-110K without EQLC1-MG	MPEQ-8110-1	500 µL

2. Invert the vial several times to mix. Vortex the vial for 10 seconds. Allow the vial to sit for 5-10 minutes and then transfer the standard to an appropriately labeled polypropylene microfuge tube.

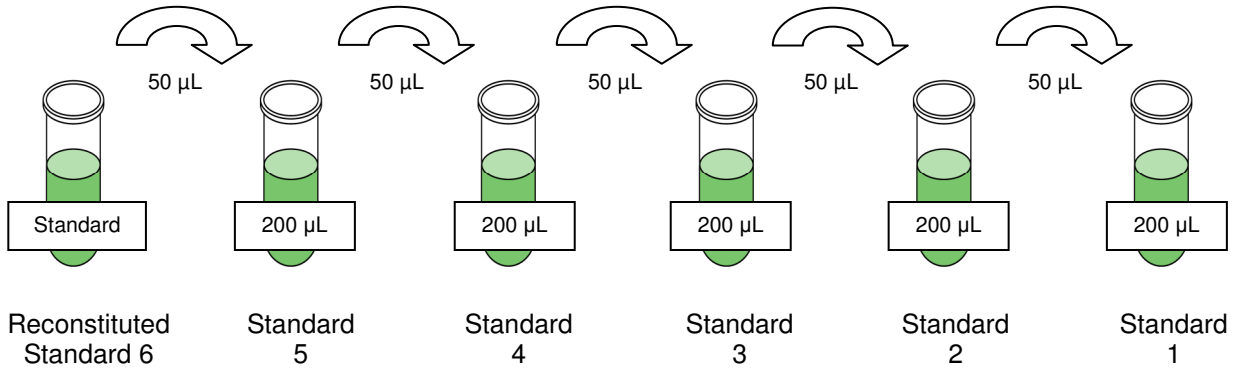
3. When using MPEQMG-110K-PX22 or MPEQMAG-110K with EQLC1-MG, combine 200 µL MPEQ-8110-1 and 200 µL MPEQ-8110-2 in a labeled polypropylene microfuge tube, this will be used as the top standard (Standard 6); any unused portion may be stored at ≤ -20°C.
4. When using MPEQMAG-110K without EQLC1-MG, MPEQ-8110-1 will be used as the top standard (Standard 6); any unused portion may be stored at ≤ -20°C.

Preparation of Working Standard

Label five polypropylene microfuge tubes as Std 5, Std 4, Std 3, Std 2 and Std 1. Add 200 µL Dilution Buffer to each of the five tubes. Prepare serial dilutions by adding 50 µL of reconstituted Standard 6 to the Std 5 tube; mix well and transfer 50 µL of standard 5 to the Std 4 tube; mix well and transfer 50 µL of standard 4 to the Std 3 tube; mix well and transfer 50 µL of standard 3 to the Std 2 tube; mix well and transfer 50 µL of standard 2 to the Std 1 tube and mix well. The 0 pM standard (Background) will be Dilution Buffer only.

Standard	Volume of Dilution Buffer to Add	Volume of Standard to Add
Original (Std 6)	250 µL	0

Standard	Volume of Dilution Buffer to Add	Volume of Standard to Add
Standard 5	200 µL	50 µL of Standard 6
Standard 4	200 µL	50 µL of Standard 5
Standard 3	200 µL	50 µL of Standard 4
Standard 2	200 µL	50 µL of Standard 3
Standard 1	200 µL	50 µL of Standard 2



NOTE: The reconstituted Peptide Standard is at the following initial concentrations for each of the analytes.

TARGET	[Peptide], Standard 6 (pM)
Csk (pY304)	1000
EGFR (pY1110/1125)	1000
EGFR total	10000
ErbB3 (pY1197/1199/1262/1276/1289/1307)	10000
ERK1/2 (pY204/187)	5000
FAK (pY397/407)	1000
FAK (pY861)	5000
FRS2 (pY436)	5000
Gab1 (pY285/307/317)	5000
Gab2 (pY266)	5000
Gab2 (pY614)	5000
Gab2 (pY584)	1000
HER2 (pY1023)	5000
PKCμ (pY502)	5000
Pl3Kp110δ (pY485)	1000
PLCγ1 (pY1253)	1000
PLCγ2 (pY1197/1217)	1000
Shc (pY349/350)	5000
SPRY2 (pY55)	5000
STAT3 (pY705)	1000
TAFII68 (loading control)	5000
EGFR (pY1069/1092)	5000

D. Preparation of Samples

The following protocol allows for the preparation of an appropriate amount of sample for duplicate well measurement. If triplicate or single well measurement is desired scale up or down as appropriate.

1. For each sample, in a clean tube combine 30 μL digested sample (completed cell lysate samples prepared using EpiQuant Sample Preparation Kit (MPEQ-SP) and 30 μL 1X Assay buffer.
2. Repeat for each sample to be assayed.

E. Preparation of Detection Antibody and SAPE

1. Vortex the EpiQuant EGFR Detection Antibody (25X) and the SAPE for 10 seconds.
2. In a Mixing Bottle, combine 120 μL EGFR Detection Antibody (25X) and 2.88 mL 1X Assay Buffer.
3. In a separate Mixing Bottle, combine 100 μL SAPE (25X) and 2.4 mL 1X Assay Buffer.

IMMUNOASSAY PROCEDURE

- Prior to beginning this assay, it is imperative to read this protocol completely and to thoroughly understand the Technical Guidelines.
- Allow all reagents to warm to room temperature (20-25°C) before use in the assay.
- Diagram the placement of Standards [0 (Background), 1,2,3,4,5 and 6], Controls 1 and 2, and Samples on Well Map Worksheet in a vertical configuration. (Note: Most instruments will only read the 96-well plate vertically by default.) It is recommended to run the assay in duplicate.
- If using a filter plate, set the filter plate on a plate holder at all times during reagent dispensing and incubation steps so that the bottom of the plate does not touch any surface.
- **All aspiration steps must be performed with the plate placed on a magnetic base.**

1. Add 25 uL Assay Buffer to each well of the Assay Plate. Seal and mix on a plate shaker for 10 minutes at room temperature (20-25°C).
2. Decant Assay Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times.
3. Add 25 µL of each Standard or Control into the appropriate wells. Dilution Buffer should be used for the 0 pg/mL standard (Background).
4. Add 25 µL of prepared sample into the appropriate wells.
5. Vortex Mixing Bottle and add 25 µL Mixed or Premixed Beads to each well. (Note: During addition of Beads, shake bead bottle intermittently to avoid settling.)
6. Seal the plate with a plate sealer. Wrap the plate with foil and incubate with agitation on a plate shaker for 2 hours at room temperature (20-25°C).
7. Remove fluid by aspiration. **(NOTE: DO NOT INVERT PLATE** unless using hand held magnetic separation block**.)** To avoid aspiration related bead loss, allow the plate to soak on the magnet of the plate washer for 60 seconds prior to aspiration.
8. Wash plate 2 times with 100 µL/well of Assay Buffer, removing Assay Buffer by aspiration between each wash. To avoid bead loss, allow approximately 60 seconds between dispensing of the Wash Buffer and subsequent aspiration.

Add 25 µL Assay Buffer per well

Shake 10 min, RT

Decant

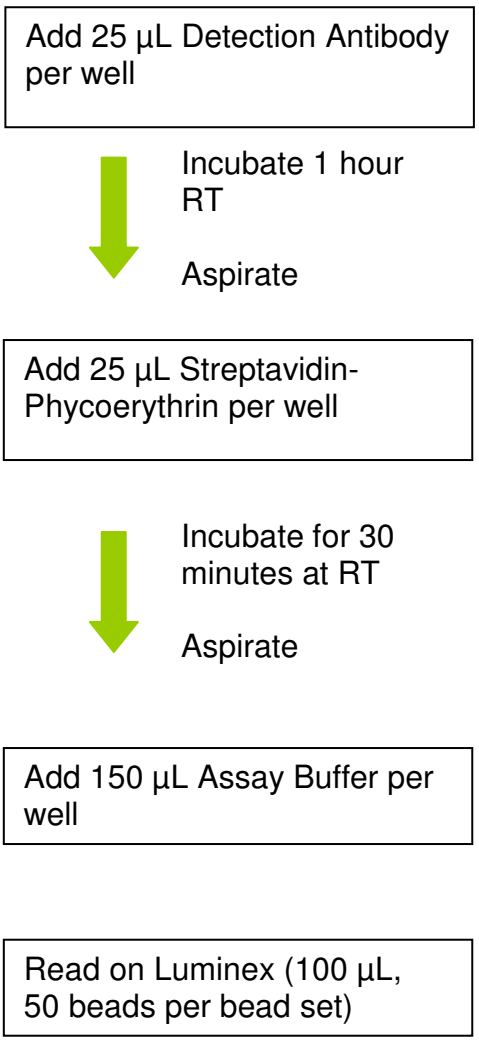
- Add 25 µL Standard or Control to appropriate wells and 25µL Dilution Buffer to background wells.
- Add 25 µL prepared samples to sample wells
- Add 25 µL Beads to each well

Incubate 2 hours at RT with shaking

Aspirate and wash 2X with 100 µL Assay Buffer

Note: If using the recommended Plate washer for magnetic beads (Bio-Tek ELx405) follow the appropriate equipment settings outlined below. . If using Hand Held Magnetic Separation Block (40-285), follow instructions included with the magnet.

9. Add 25 µL of 1X Detection Antibody into each well.
10. Seal, cover with foil, and incubate with agitation on a plate shaker for 1 hour at room temperature (20-25°C). Remove contents by aspiration after agitation. **(NOTE: DO NOT INVERT PLATE** unless using hand held magnetic separation block**.)**
11. Add 25 µL 1X Streptavidin-Phycoerythrin to each well.
12. Seal, cover with foil and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C).
13. Remove fluid by aspiration. **(NOTE: DO NOT INVERT PLATE** unless using hand held magnetic separation block**.)**
14. Add 150 µL of Assay Buffer to all wells. Resuspend the beads on a plate shaker for 5 minutes.
15. Analyze plate on Luminex, 200™, HTS, FLEXMAP 3D™ or MAGPIX®.
16. Save and analyze the Median Fluorescent Intensity (MFI) data using a weighted 5-parameter logistic or spline curve-fitting method for calculating peptide concentrations in samples.



EQUIPMENT SETTINGS

Bio-Tek ELx405:

The general recommended wash protocol (Link Protocol) is as follows:

Soak Program:	Wash Program:
Soak →	Aspirate→Dispense→Soak→Aspirate→Dispense→Soak→Aspirate

1.) Soak program:
   1. Soak duration: 60 sec
   2. Shake before soak?: NO

2.) Wash program:

Method:
   1. Number of cycles: 2
   2. soak/shake: YES
   3. Soak duration: 60 sec
   4. Shake before soak: NO
   5. Prime after soak: NO

Dispense:
   1. Dispense volume: 100 µL/well
   2. Dispense flow rate: 5
   3. Dispense height: 130 (16.51 mm)
   4. Horizontal disp pos: 00 (0 mm)
   5. Bottom Wash first?: NO
   6. Prime before start?: NO

Aspiration:
   1. Aspirate height: 35 (4.445 mm)
   2. Horizontal Asp Pos: 30 (1.372 mm)
   3. Aspiration rate: 06 (15.0 mm/sec)
   4. Aspiration delay: 0
   5. Crosswise Aspir: NO
   6. Final Aspir: YES
   7. Final Aspir delay: 0 (0 msec)

3.) Link program: (**Note:** this is the program to use during actual plate washing between Standard and Sample incubation and Detection incubation).
Link together the Soak and Wash programs outlined above.

Note: After the final aspiration, there will be approximately 25 µl of residual Wash Buffer in each well. This is expected when using the BioTek Plate washer and this volume does not need to be aspirated from the plate.

If using an automatic plate washer other than BioTek ELx405, please refer to the manufacturer's recommendations for programming instructions.

Luminex 200™, HTS, FLEXMAP 3D™ and MAGPIX®with xPONENT software:

These specifications are for the Luminex 200™, Luminex HTS, Luminex FLEXMAP 3D™ and Luminex MAGPIX® with xPonent software. Luminex instruments with other software (e.g. MasterPlex, StarStation, LiquiChip, Bio-Plex, LABScan100) would need to follow instrument instructions for gate settings and additional specifications from the vendors for reading Luminex Magnetic Beads.

For magnetic bead assays, the Luminex 200™ and HTS instruments must be calibrated with the xPonent 3.1 compatible Calibration Kit (Millipore Cat.# 40-275) and performance verified with the Performance Verification Kit (Millipore Cat. # 40-276). The Luminex FLEXMap 3D™ instrument must be calibrated with the FLEXMAP 3D™ Calibrator Kit (Millipore cat#40-028) and performance verified with the FLEXMAP 3D™ Performance Verification Kit (Millipore cat#40-029). The Luminex MAGPIX® instrument must be calibrated with the MAGPIX® Calibration Kit (Millipore cat# 40-049 and performance verified with the MAGPIX® Performance Verification Kit (Millipore cat# 40-050).

NOTE: These assays cannot be run on Luminex 100™ instruments or any instruments using Luminex IS 2.3 or Luminex 1.7 software.

The Luminex probe height must be adjusted to the plate provided in the kit. Please use Cat.# MAG-PLATE, if additional plates are required for this purpose.

Events:	50 per bead	
Sample	100 µL	
Gate Settings:	8,000 to 15,000	
Reporter	Default (low PMT)	
Time	60 seconds	
Bead		
	Csk (pY304)	13
	EGFR (pY1110/1125)	15
	EGFR total	19
	ErbB3 (pY1197/1199/1262/1276/1289/1307)	25
	ERK1/2 (pY204/187)	27
	FAK (pY397/407)	29
	FAK (pY861)	33
	FRS2 (pY436)	35
	Gab1 (pY285/307/317)	37
	Gab2 (pY266)	39
	Gab2 (pY614)	43
	Gab2 (pY584)	45
	HER2 (pY1023)	47
	PKCµ (pY502)	57
	Pl3Kp110δ (pY485)	61
	PLCγ1 (pY1253)	63
	PLCγ2 (pY1197/1217)	65
	Shc (pY349/350)	67
	SPRY2 (pY55)	73
	STAT3 (pY705)	75
	TAFII68 (loading control)	77
	EGFR (pY1069/1092)	78

QUALITY CONTROLS

The ranges for each analyte in Quality Control 1 and 2 are provided on the card insert or can be located at the Millipore website www.millipore.com/techlibrary/index.do using the catalog number as the keyword.

ASSAY CHARACTERISTICS

Cross-Reactivity

No significant cross-reactivity was observed between the antibodies and/ or non-specific analytes in this panel.

Precision

Intra-assay precision is generated from the mean of the %CV's from 8 reportable results across two different concentrations of target peptide in one experiment. Inter-assay precision is generated from the mean of the %CV's from two reportable results, each for two different concentrations of target peptide across 6 different experiments. Intra-assay precision for all analytes ranges from **1.5%** to **17.1%** and Inter-assay precision for all analytes ranges from **2.8%** to **21.6%**.

Accuracy

Spike-Recovery values are generated from the mean percent recovery of 3 levels of spiked standards ranging from 8pM to 2000pM in sample matrix in 8 experiments. Spike-recovery values for all analytes range from **80.5%** to **115.6%**.

Assay Sensitivity (minimum detectable concentrations, pM)

MinDC: Minimum Detectable Concentration is calculated by STATLIA Immunoassay Analysis Software. It measures the true limits of detection for an assay by mathematically determining what the empirical MinDC would be if an infinite number of standard concentrations were run for the assay under the same conditions. MinDC values were determined from 6 different experiments.

MinDC < 0.32	MinDC = 0.33-1.6	MinDC = 1.7-3.1	MinDC > 3.2
Csk (pY304)	EGFR (pY1069/1092)	Gab2 (pY266)	ErbB3 (pY1197/1199/1262/1276/1289/1307)
EGFR (pY1110/1125)	EGFR total	PKCμ (pY502)	ERK1/2 (pY204/187)
FAK (pY397/407)	FRS2 (pY436)		FAK (pY861)
PLCγ1 (pY1253)	Gab1 (pY285/307/317)		Gab2 (pY584)
PLCγ2 (pY1197/1217)	Gab2 (pY614)		Shc (pY349/350)
STAT3 (pY705)	HER2 (pY1023)		SPRY2 (pY55)
	Pl3Kp110δ (pY485)		TAFII68 (loading control)

Natural Sample Response

Prepared cell lysates (MPEQ-LY001/2/3/4/5) were assayed using the MPEQMAG-110K kit. Results are tabulated below; values are the average of triplicate measurements.

TARGET	TARGET CONCENTRATION IN CONTROL LYSATES (pM)				
	MPEQ-LY001	MPEQ-LY002	MPEQ-LY003	MPEQ-LY004	MPEQ-LY005
Csk (pY304)	**0.32**	**0.16**	**0.04**	**0.43**	**0.73**
EGFR (pY1110/1125)	**3.01**	**2.56**	**1.15**	**131.04**	**106.42**
EGFR total	**8.01**	**256.19**	**1.48**	**204.71**	**191.96**
ErbB3 (pY1197/1199/1262/1276/1289/1307)	**51.49**	**22.49**	**5.70**	**70.51**	**66.07**
ERK1/2 (pY204/187)	**5.96**	**<1.04**	**93.16**	**39.99**	**103.28**
FAK (pY397/407)	**3.42**	**2.73**	**1.30**	**28.40**	**7.00**
FAK (pY861)	**6.22**	**7.91**	**49.36**	**305.02**	**10.34**
FRS2 (pY436)	**12.05**	**4.07**	**1.78**	**7.93**	**21.07**
Gab1 (pY285/307/317)	**6.86**	**2.82**	**3.41**	**5.66**	**7.34**
Gab2 (pY266)	**11.52**	**5.41**	**3.24**	**6.68**	**21.75**
Gab2 (pY614)	**2.77**	**1.73**	**2.88**	**1.23**	**0.94**
Gab2 (pY584)	**12.26**	**11.41**	**12.84**	**15.75**	**23.57**
HER2 (pY1023)	**22.87**	**9.13**	**3.81**	**124.86**	**29.31**
PKCμ (pY502)	**10.55**	**<0.39**	**<0.39**	**0.80**	**13.73**
Pl3Kp110δ (pY485)	**2.47**	**1.30**	**0.75**	**2.39**	**4.95**
PLCγ1 (pY1253)	**0.13**	**0.26**	**14.01**	**1.25**	**3.35**
PLCγ2 (pY1197/1217)	**2.10**	**0.70**	**16.86**	**3.06**	**2.73**
Shc (pY349/350)	**9.86**	**30.31**	**135.72**	**238.01**	**605.25**
SPRY2 (pY55)	**21.95**	**2.40**	**2.75**	**22.38**	**19.39**
STAT3 (pY705)	**2.11**	**0.61**	**0.25**	**1.39**	**2.50**
TAFII68 (loading control)	**69.65**	**134.24**	**68.24**	**124.90**	**130.02**
EGFR (pY1069/1092)	**14.73**	**3.74**	**3.13**	**39.39**	**55.21**

MPEQ-LY001- EpiQuant Jurkat: Unstimulated Lysate
MPEQ-LY002- EpiQuant A431: Unstimulated Lysate
MPEQ-LY003- EpiQuant Jurkat: H_2O_2 Stimulated Lysate
MPEQ-LY004- EpiQuant A431: H_2O_2 Stimulated Lysate
MPEQ-LY005- EpiQuant A431: EGF Stimulated Lysate

TROUBLESHOOTING GUIDE

Problem	Probable Cause	Solution
Insufficient Bead Count	Plate Washer aspirate height set too low	Adjust aspiration height according to manufacturers instructions.
	Bead mix prepared inappropriately	Sonicate bead vials and vortex just prior to adding to bead mix bottle according to protocol. Agitate bead mix intermittently in reservoir while pipetting this into the plate.
	Samples cause interference due to particulate matter or viscosity	See above. Also sample probe may need to be cleaned with Alcohol flush, Back flush and washes; or if needed probe should be removed and sonicated.
	Probe height not adjusted correctly	When reading the assay on Luminex 200™, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 4 alignment discs. When reading the assay on FLEXMAP 3D™, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 1 alignment disc. When reading the assay on MAGPIX, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 2 alignment disc.
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using sealer appropriately, and pipeting with Multichannel pipets without touching reagent in plate.
	Matrix used has endogenous analyte or interference	Check matrix ingredients for cross reacting components (e.g. interleukin modified tissue culture medium).
	Insufficient washes	Increase number of washes.
Beads not in region or gate	Luminex not calibrated correctly or recently	Calibrate Luminex based on Instrument Manufacturer's instructions, at least once a week or if temperature has changed by >3^{o}C.
	Gate Settings not adjusted correctly	Some Luminex instruments (e.g. Bioplex) require different gate settings than those described in the Kit protocol. Use Instrument default settings.
	Wrong bead regions in protocol template	Check kit protocol for correct bead regions or analyte selection.
	Incorrect sample type used	Samples containing organic solvents or if highly viscous should be diluted or dialyzed as required.
	Instrument not washed or primed	Prime the Luminex 4 times to rid of air bubbles, wash 4 times with sheath fluid or water if there is any remnant alcohol or sanitizing liquid.
	Beads were exposed to	Keep plate and bead mix covered with dark

	light	lid or aluminum foil during all incubation steps.
Signal for whole plate is same as background	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue.
	Streptavidin-Phycoerythrin was not added	Add Streptavidin-Phycoerythrin according to protocol. If Detection Antibody has already been removed, sensitivity may be low.
Low signal for standard curve	Detection Antibody may have been removed prior to adding Streptavidin Phycoerythrin	May need to repeat assay if desired sensitivity not achieved.
	Incubations done at inappropriate temperatures, timings or agitation	Assay conditions need to be checked.
Signals too high, standard curves are saturated	Calibration target value set too high	With some Luminex Instrument (e.g. Bio-plex) Default target setting for RP1 calibrator is set at High PMT. Use low target value for calibration and reanalyze plate.
	Plate incubation was too long with standard curve and samples	Use shorter incubation time.
Sample readings are out of range	Samples contain no or below detectable levels of analyte	If below detectable levels, it may be possible to use higher sample volume. Check with tech support for appropriate protocol modifications.
	Samples contain analyte concentrations higher than highest standard point.	Samples may require dilution and reanalysis for just that particular analyte.
	Standard curve was saturated at higher end of curve.	See above.
High Variation in samples and/or standards	Multichannel pipet may not be calibrated	Calibrate pipets.
	Plate washing was not uniform	Confirm all reagents are removed completely in all wash steps.
	Samples may have high particulate matter or other interfering substances	See above.
	Plate agitation was insufficient	Plate should be agitated during all incubation steps using a vertical plate shaker at a speed where beads are in constant motion without causing splashing.
	Cross well contamination	Check when reusing plate sealer that no reagent has touched sealer. Care should be taken when using same pipet tips that are used for reagent additions and that pipet tip does not touch reagent in plate.
FOR FILTER PLATES ONLY		
Filter plate will not vacuum	Vacuum pressure is insufficient	Increase vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds.
	Samples have insoluble particles	Centrifuge samples just prior to assay setup and use supernatant.
	High lipid concentration	After centrifugation, remove lipid layer and

		use supernatant.
Plate leaked	Vacuum Pressure too high	Adjust vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds. May need to transfer contents to a new (blocked) plate and continue.
	Plate set directly on table or absorbent towels during incubations or reagent additions	Set plate on plate holder or raised edge so bottom of filter is not touching any surface.
	Insufficient blotting of filter plate bottom causing wicking	Blot the bottom of the filter plate well with absorbent towels after each wash step.
	Pipette touching plate filter during additions	Pipette to the side of plate.
	Probe height not adjusted correctly	Adjust probe to 3 alignment discs in well H6.
	Sample too viscous	May need to dilute sample.

REPLACEMENT REAGENTS	Cat #
EpiQuant EGFR Panel Standard 1	MPEQ-8110-1
EpiQuant EGFR Panel Standard 2	MPEQ-8110-2
EpiQuant EGFR Panel Quality Controls 1 and 2	MPEQ-6110
Set of two 96-Well Plates with 2 Sealers	MAG-PLATE
EpiQuant Assay Buffer (10X)	MPEQ-AB
EpiQuant Dilution Buffer	MPEQ-DB
EpiQuant EGFR Detection Antibody (25X)	MPEQ-1110
SAPE (25X)	45-001D

Antibody-Immobilized Magnetic Beads

Target	Bead #	Cat. #
Csk (pY304)	13	EQCSK-Y304MG
EGFR (pY1110/1125)	15	EQEGFR-Y1110MG
EGFR (pY1069/1092)	78	EQEGFR-Y1069MG
EGFR total	19	EQEGFR-TOTALMG
ErbB3 (pY1197/1199/1262/1276/1289/1307)	25	EQERBB3-Y1197MG
ERK1/2 (pY204/187)	27	EQERK1/2-Y204MG
FAK (pY397/407)	29	EQFAK-Y397MG
FAK (pY861)	33	EQFAK-Y861MG
FRS2 (pY436)	35	EQFRS2-Y436MG
Gab1 (pY285/307/317)	37	EQGAB1-Y285MG
Gab2 (pY266)	39	EQGAB2-Y266MG
Gab2 (pY584)	43	EQGAB2-Y584MG
Gab2 (pY614)	45	EQGAB2-Y614MG
HER2 (pY1023)	47	EQHER2-Y1023MG
PKCμ (pY502)	57	EQPKCM-Y502MG
Pl3Kp110δ (pY485)	61	EQPI3K-Y485MG
PLCγ1 (pY1253)	63	EQPLCG1-Y1253MG
PLCγ2 (pY1197/1217)	65	EQPLCG2-Y1197MG
Shc (pY349/350)	67	EQSHC-Y349MG
SPRY2 (pY55)	73	EQSPRY2-Y55MG
STAT3 (pY705)	75	EQSTAT3-Y705MG
TAFII68 (loading control)	77	EQLC1-MG

Premixed 22Plex Beads EQ110MAG-PMX22

ORDERING INFORMATION

To place an order:

To assure the clarity of your custom kit order, please FAX the following information to our customer service department:

- Your name, telephone and/or fax number
- Customer account number
- Shipping and billing address
- Purchase order number
- Catalog number and description of product
- Quantity of kits
- Selection of MILLIPLEX *MAP* Analytes

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Toll Free US: (800) MILLIPORE

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St. Charles, Missouri 63304 U.S.A.

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WELL MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 pM Standard (Background)	Standard 4	QC-2 Control									
B	0 pM Standard (Background)	Standard 4	QC-2 Control									
C	Standard 1	Standard 5	Sample 1									
D	Standard 1	Standard 5	Sample 1									
E	Standard 2	Standard 6	Sample 2									
F	Standard 2	Standard 6	Sample 2									
G	Standard 3	QC-1 Control	Etc.									
H	Standard 3	QC-1 Control										