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Multiplex ELISA Kit For Mouse Cytokine Panel 3 (4-Plex)

Catalog number: MEK2005

For detection of multiple analytes using one single assay.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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Multiplex ELISA Kit For Mouse Cytokine Panel 3 (4-Plex)

Catalog Number: MEK2005
Storage and Shelf Life : Store unopened kit at 2-8°C until kit expiration. Avoid exposure to UV light.

Product Overview

The Multiplex ELISA Kit For Mouse Cytokine Panel 3 (4-Plex) is a fully quantitative ELISA-based chemiluminescent assay allowing concurrent measurement of four biomarkers or analytes (IL-2, IL-4, IL-6, TNFalpha).

Each kit contains a 96-well plate, with each well featuring the relevant biomarker panel, and all the reagents required to perform testing. Multiplex plates are built by absorbing four distinct capture antibodies in a defined array to the bottom of each well. Our high-quality reagents help ensure the accuracy of your results.

Using just 25 µl of sample per well, up to 80 samples can be assayed for all four markers in the panel in 5.5 hours. Multiplex ELISA Kit For Mouse Cytokine Panel 3 (4-Plex) provides scientists with an easy-to-use and cost-effective means of generating a cytokine profile for each sample.

Assay Ranges

Analyte	Assay Type	Calibrator Range	Upper Limit of Quantification (ULOQ)	Lower Limit of Quantification (LLOQ)	Limit of Detection	Precision (Inter-assay)	Precision (Intra-assay)	Average Linearity
IL-2	Sandwich	360 - 0.49 (pg/mL)	161 (pg/mL)	0.41 (pg/mL)	0.25 (pg/mL)	5.1%	6.6%	88%
IL-4	Sandwich	400 - 0.55 (pg/mL)	126 (pg/mL)	0.32 (pg/mL)	0.27 (pg/mL)	6.2%	6.6%	98%
IL-6	Sandwich	504 - 0.69 (pg/mL)	819 (pg/mL)	2.09 (pg/mL)	0.35 (pg/mL)	7.3%	6.1%	106%
TNFalpha	Sandwich	429 - 0.59 (pg/mL)	217 (pg/mL)	0.55 (pg/mL)	0.29 (pg/mL)	6.9%	5.2%	104%

Kit Components

Each kit contains a 96-well plate, featuring the relevant biomarker panel in each well, and all reagents required to perform testing.

Reagents Include:

Calibrator: Lyophilized, recombinant antigens in a buffered protein base
Detection Mix: Lyophilized, 6 mL/vial of biotinylated antibodies in a buffered protein solution with preservatives
Sample Diluent: Liquid, 10 mL/vial of a buffered protein solution
Substrate A: Liquid, 3.5 mL/vial
Substrate B+: Liquid, 3.5 mL/vial
Streptavidin-HRP: Liquid, 6 mL/vial of streptavidin-conjugated horseradish peroxidase
Wash Buffer Concentrate (20X): Liquid, 50 mL/vial of a concentrated solution of buffered surfactant
Plate Seals (3): Adhesive strips

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Assay Principle

This multiplex assay is based on the 96 well plate sandwich enzyme immunoassay technique for the measurement of multiple analytes. For each analyte, two different antibodies targetting different epitope are used, one arrayed to the bottom of the plate and one for detection of the captured analytes. Samples or calibrators are pipetted into wells of a 96 well plate and the analytes of interests are immobilized by their corresponding capture antibodies. After washing away any unbound proteins, a mixture that contains biotinylated analyte specific antibodies is added. The biotinylated antibodies complete the sandwich for each specific arrayed analyte. After washing away unbound biotinylated antibody, streptavidin-horseradish peroxidase (SHRP) is added. Following an additional wash, the amount of SHRP remaining on each location of the array is proportional to the amount of analytes initially captured. The amount of conjugated enzyme on each location of the array is measured with the addition of a chemiluminescent substrate.

Sample Collection And Storage

The sample collection and storage conditions are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Cell culture supernates should contain at least 1% fetal calf serum for stability of the proteins. Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Before Starting

1. Read all instructions before beginning test.

2. For research use only. Not for use in diagnostic procedures.

3. The kit should not be used beyond the expiration date on the kit label.

4. **This kit is sensitive to saliva. Wear a mask during preparation and running the kit.**

5. If running multiple kits, a calibration curve must be included for each 96-well plate. The curve from one plate cannot be used to calculate sample values from other plates.

6. Do not mix or substitute reagents with those from other kits or lots.

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7. All products are carefully validated, however due to the variability encountered in biological buffers and sample matrices, the possibility of interference or sample matrix effects cannot be excluded.

8. Warning: The calibrator contains components of human origin. These components have been tested at the donor level and found negative for HBsAg, HIV-1 and HIV-2 antibodies, and HCV. However, consider all materials as potentially infectious and use only approved guidelines for the proper handling and disposal of infectious material.

Best Practices

Dilute all samples at least 1:2 (one part sample to one part diluent) with the provided sample diluent to prevent false positives, and mix thoroughly.

Load all calibrators and samples into the microplate within 10 minutes of each other.

Be exact with incubation times, particularly the streptavidinhorseradish peroxidase (SHRP) incubation.

Be exact when mixing Substrate A and B and mix thoroughly.

Do not allow the plate to dry out between steps.

Do not allow the substrate or SHRP to be exposed to UV light, as this may degrade it.

Materials Needed But Not Included

1. Multichannel pipette (20-200 uL) and/or a single channel pipette (20- 200 uL) and tip

2. Polypropylene tubes or polypropylene 96-well plate(s) for sample and calibrator preparation

3. Microplate shaker capable of 500 RPM.

4. Deionized water

5. Microplate washer

6. Graduated cylinder for the preparation of wash buffer

Assay Preparation

1. Set the plate shaker to 500 RPM.

2. Prepare the Wash Buffer: Place 50 mL of the 20X concentrate into 950 mL deionized water, and mix thoroughly.

3. Prepare Calibrator: Reconstitute using Sample Diluent with the volume on the Product Card which accompanies the kit. Allow Calibrator to sit for

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5 minutes. Mix thoroughly.

4. Allow Substrate A and B to come to room temperature (20-25°C). Fifteen minutes prior to use, combine 3 mL of Substrate A with 3 mL of Substrate B, and mix gently. Do not expose to UV light. Store at room temperature (20-25°C) after mixing.

Assay Procedure

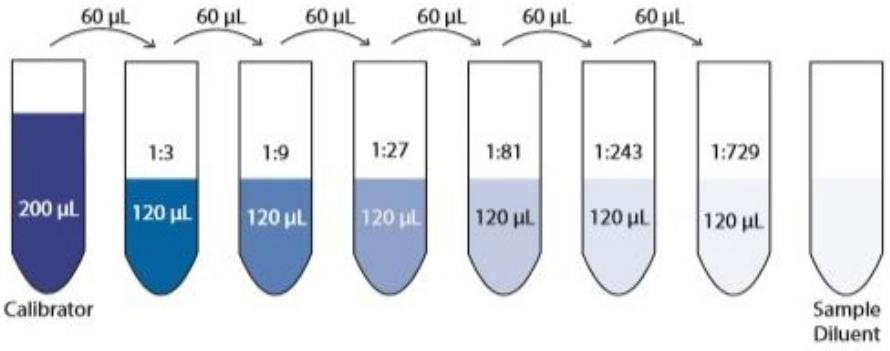
This assay is saliva sensitive. Wear a mask when running this kit. Allow all reagents to equilibrate to room temperature (20-25°C) before use and prepare as directed by the previous sections. It is recommended that all calibrators, samples, and controls be assayed in duplicate.

1. Using the previously reconstituted calibrator, prepare an 8-point calibration curve (7 points plus 1 blank) in either polypropylene tubes or a polypropylene 96-well plate.

a. Pipette 200 uL of prepared calibrator into the first tube or well.

b. Place 120 uL of prepared sample diluent into the other seven tubes or wells.

c. Transfer 60 uL of the undiluted prepared calibrator from the first tube into the second, mix thoroughly, and repeat the transfer from tube to tube for 5 more points, leaving the last tube without any prepared calibrator. This process is diagrammed above. The undiluted prepared calibrator serves as the high point of the standard curve. The prepared sample diluent serves as the negative.



2. Prepare plasma samples by diluting 1:2 (one part sample to one part prepared sample diluent) with enough prepared sample diluent to have 50 uL per well in either polypropylene tubes or a polypropylene 96-well plate.

Note: If you anticipate that your analyte concentrations will be greater than the high point of the calibration curves as reported on the product card included in the kit, use the prepared sample diluent to dilute your samples further.

3. Add 50 uL per well of the calibration curve and samples by pre-wetting the pipette tips three times, drawing up the liquid into your pipette and then dispensing back into the original vessel, aspirating 50 uL and dispensing into the 96-well plate. Load all samples and calibration curve to the plate within ten minutes.

4. Cover the plate with a plate seal provided, and shake on a plate shaker set to 500 RPM for one hour at room temperature (20-25°C).

5. Wash the plate three times

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6. Add 50 uL per well of Detection Mix, cover with a new plate seal, and return to the plate shaker set to 500 RPM for one hour at room temperature (20-25°C).

7. Wash the plate three times (see Appendix A).

8. Add 50 uL per well of Streptavidin-HRP 1X, cover with a new plate seal, and return to the plate shaker set to 500 RPM for 15 minutes at room temperature (20-25°C).

9. Wash the plate six times (see Appendix A).

10. Add 50uL per well of previously prepared substrate. Wait no longer than 15 minutes to commence imaging. Note: If imaging cannot commence immediately, protect the plate from drying for up to 15 minutes by dispensing 100 uL of wash buffer into each well of the plate prior to adding the mixed substrate. When ready to image, remove the wash buffer from the plate and add the substrate.

11. Set aperture (f-stop) to the lowest value to a minimum of 2, set binning to the lowest value.

12. Adjust zoom so the plate takes up about 80% of the field of view in the imaging software.

13. Adjust focus so that individual spots on the Calibration Kit mouse pad are clear and visible in the imaging software.

14. Place the plate in the compatible imager, take image and export result in grayscale images using “Export For Analysis”, “Export Raw Data,” etc. Choose a lossless file type, such as TIFF and at least 12-bit depth output.

15. Dispose of all used and unused materials. Disposal of waste may differ from country to country. Please refer to local disposal rules.

16. Send the result data to support@bosterbio.com for analysis. If you would like to analyze the data internally, please contact us to set up.

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