

# FIDIS™ HUMAN NEUROSCIENCE CYTOKINE CORE PANEL 4-PLEX KIT

English

REF

MX HN0401



*For Research Use Only*

## INTRODUCTION

Advances in the field of cell biology have defined a complex and interdependent set of extracellular and intracellular signaling molecules that control normal cell function. Perturbations in signaling pathways may be important indicators, and possibly the root cause, of many diseases. Therefore, there is growing interest among clinicians as well as drug discovery groups in monitoring multiple components of signaling pathways simultaneously. Solid phase multiplex protein assays have become the primary tools of choice in these studies as they maximize efficiency by permitting several analytes to be profiled within individual samples.

bmd's Multiplex Bead Immunoassays are solid phase protein assays which use spectrally encoded antibody-conjugated beads as the solid support. The spectral beads are suitable for use in singleplex assays or may be mixed for multiplex assays according to the researcher's needs. Each assay is carefully designed and tested to assure that sensitivity, range and correlation are maximized. The assay is performed in a 96 well plate format and analyzed with a FIDIS™ instrument which monitors the spectral properties of the capture beads while simultaneously measuring the quantity of associated fluorophore. Standard curves generated with this assay system extend over several orders of magnitudes of concentrations, while the sensitivity and quantitation of the assays are comparable to ELISAs (Enzyme Linked-Immuno-Sorbent Assays). Assay standards are calibrated to NIBSC reference preparations, when available, to assure accurate and reliable results.

This kit provides a series of combined reagents for the simultaneous measurement of IL-1β, IL-6, IL-8, and TNF-α. The reagents included in this kit have been optimized for use with cerebrospinal fluid (CSF) and tissue culture medium samples. The FIDIS™ Human Neuroscience Cytokine Core Panel may be run alone, or combined with other Neuroscience antibody bead kits available from bmd, for higher level multiplexing.

For a current listing of available bmd Antibody Multiplex Bead Immunoassay and reagents, please contact us.

**This kit has been configured for research use only and is not to be used in diagnostic procedures.**

**Read entire protocol before use.**

## PRINCIPLE OF THE METHOD

bmd's Neuroscience Bead Immunoassays are solid phase sandwich immunoassays, which are designed to be analyzed with a FIDIS™ instrument. The spectral properties of 100 distinct bead regions can be monitored with the FIDIS™ instrument, a capability that affords this assay system the potential for measuring up to 100 different analytes in a single sample.

- Beads of defined spectral properties conjugated to analyte specific capture antibodies are first pipetted into the wells of a filter plate and then washed and aspirated.
- Next, detector antibody is pipetted into the wells, followed immediately by standard or sample.

- The beads, antibody, plus standard or sample are then incubated together for three hours at room temperature. At the end of this incubation, the wells are aspirated, then washed four times.
- Next, R-Phycoerythrin (RPE) conjugate is pipetted into the wells and incubated for 30 minutes. During this incubation, the RPE conjugate binds to detector antibody associated with the bead. At the end of this incubation, the wells are washed four times.
- The beads are then resuspended in wash buffer, and the spectral properties of the beads are monitored with the FIDIS™ instrument. By monitoring the spectral properties of the beads and the amount of associated R-Phycoerythrin fluorescence, the concentration of one or more analytes can be determined.

## REAGENTS PROVIDED

**Note:** Store all reagents at +2°C / +8°C.

	MX HN0401
96 well Filter Plate (Extra plates can be purchased from Millipore/Fisher Cat. # MSBVN-1250 or Pall Cat.# S5039)	1 plate
Hu Neuroscience Cytokine Core Panel Bead Concentrate (10x). Contains 7.5 mM sodium azide; 0.25 mL per vial.	1 vial
Hu Neuroscience Cytokine Core Panel Detector Antibody Concentrate (10x). Contains 3.3 mM thymol; 0.5 mL per vial.	1 vial
Neuroscience Cytokine Core Panel Standard.	2 vials
Wash Solution Concentrate (20x); 15 mL per bottle.	1 bottle
Assay Diluent. Contains 15 mM sodium azide; 15 mL per bottle.	1 bottle
Detector Antibody Diluent. Contains 3.3 mM thymol; 12 mL per bottle.	1 bottle
RPE Conjugate Concentrate (10x). Contains 15 mM sodium azide; 1 mL per vial.	1 vial
RPE Diluent. Contains 15 mM sodium azide; 15 mL per bottle.	1 bottle

**Disposal Note:** This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

## MATERIAL REQUIRED BUT NOT PROVIDED

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- FIDIS™ system. Please contact [bmd](#) for instrument and software placement services.
- [bmd](#) filtration manifold for bead washing.
- [bmd](#) data analysis and graphing software program.
- Sonicating water bath (e.g., Cole Parmer, Cat. # 08849-00).
- Orbital shaker (e.g., Fisher, Cat. # 14-271-9).
- Calibrated, adjustable, precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable.)
- Distilled or deionized water.
- Glass or plastic tubes.
- Beakers and graduated cylinders in various sizes.
- Aluminum foil.
- Extra filter plate covers.

## SAFETY

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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.

## PROCEDURAL NOTES / LAB QUALITY CONTROL

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1. The **FIDIS™ Human Neuroscience Cytokine Core Panel** may be combined with other Neuroscience Antibody Bead Kits for higher level multiplexing. See the **INFORMATION SHEET and APPENDIX I** for further information. To ensure that several Antibody Bead Kits are compatible in a multiplexed assay, verify that the bead region for each analyte (stated on the **INFORMATION SHEETS** in the Antibody Bead Kits used) is unique.
2. Do not freeze any component of this kit. When not in use, kit components should be stored at +2°C to +8°C. All reagents should be brought to room temperature before use.
3. **The fluorescent beads are light-sensitive.** Protect the beads from light to avoid photobleaching of the embedded dye. Aluminum foil should be used to cover test tubes used in the assay. Filter plates containing beads should be shielded with an aluminium foil-wrapped plate cover. The amber vial does not provide full protection, and should therefore be kept covered in the box when not in use.
4. Do not expose beads to organic solvents.
5. Do not place filter plates on absorbent paper towels during loading or incubations, as liquid will be lost due to wicking. An extra plate cover serves as a good surface upon which to rest the filter plate. Following plate washing, excess liquid may be blotted from the bottom of the plate by pressing the plate on clean paper towels.
6. Do not use reagents after kit expiration date.
7. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
8. Do not invert the filter plates during the assay. The filter plates are designed to be used in conjunction with a vacuum manifold (DO NOT EXCEED 5 inches Hg) and emptied from the bottom.
9. When pipetting reagents, maintain a consistent order of addition from well-to-well. This method ensures equal incubation times for all wells.
10. Avoid touching the filter plate membrane with pipette tips to prevent tearing.

## PREPARATION OF WASH SOLUTION

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The *Wash Solution Concentrate* is provided as a 20x concentrate. Upon storage at +2°C to +8°C, a precipitate may form. If this occurs, warm the 20x *Wash Solution Concentrate* to +37°C and vortex until the precipitate is dissolved.

To prepare the Working Wash Solution for use with a 96 well plate, transfer the entire contents of bottle to a 500 mL container and add 285 mL of deionized water. If not using an entire 96 well plate, smaller volumes of 1x Working Wash Solution can be made by mixing 1 part of 20x concentrate with 19 parts deionized water.

The Working Wash Solution is stable for up to two weeks when stored at +2°C to +8°C.

## DIRECTIONS FOR WASHING

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Incomplete washing will adversely affect assay outcome. All washing must be performed with the Wash Solution provided. All phases of the assay, including incubation steps, washing steps, and loading the beads into the FIDIS™ instrument, are performed in the filter bottom plate provided. Unused wells may be left dry during the assay and used at a later time.

1. To wash the beads, place the filter plate on the vacuum manifold and aspirate the Working Wash Solution with gentle vacuum. The vacuum setting should be adjusted so 3 seconds are required to empty 0.2 mL solution from the wells. (DO NOT EXCEED 5 inches Hg) Excessive vacuum can cause the membrane to tear, resulting in antibody bead loss. Vacuum surge should be prevented by opening and adjusting the vacuum on the manifold before placing the plate on the surface.
2. If solution remains in the wells during vacuum aspiration **DO NOT DETACH THE BOTTOM OF THE 96 WELL FILTER PLATE**. In some cases, minor clogs in the filter plate may be dislodged by carefully pressing the bottom of the plate under the clogged well with the pointed end of a 15 mL plastic conical tube.
3. Following the final aspiration step, gently blot the bottom of the filter plate on clean paper towels to remove residual liquid.

## PREPARATION OF SAMPLE

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1. CSF and tissue culture medium samples have been evaluated with this assay.
2. Samples should be collected in pyrogen/endotoxin-free polypropylene tubes.
3. Samples should be analyzed shortly after collection or frozen. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
4. All samples need to be clarified by centrifugation (14,000 rpm for 10 minutes) and/or filtered prior to analysis to prevent clogging of the filter plates.
5. Samples with concentrations that exceed the standard curve should be diluted in *Assay Diluent* (CSF samples) or appropriate tissue culture medium (tissue culture medium samples) and reanalyzed.

## REAGENT PREPARATION AND ASSAY PROCEDURE

Prior to starting the assay, prepare standards, antibody conjugated beads, and detector antibody in accordance with instructions below, then proceed to **Assay Procedure**.

### ● Preparation of Standard

Reconstitute the protein standard within one hour of performing the assay. All standards are calibrated to NIBSC preparations, when available. Additional standards are available from [bmd](#).

The concentrations of the protein components of the standard are indicated on the lot-specific **INFORMATION SHEET** included with the Antibody Bead Kit(s) used.

Standard dilutions may be performed in glass or plastic tubes.

- When assaying CSF samples, reconstitute the standard with the *Assay Diluent* provided.
- When assaying tissue culture medium samples, reconstitute the standard with a mixture of 50% *Assay Diluent* and 50% complete tissue culture medium.

For example, when the sample type is RPMI containing 5% FBS, the standards should be reconstituted in a mixture composed of 50% *Assay Diluent* and 50% RPMI containing 5% FBS.

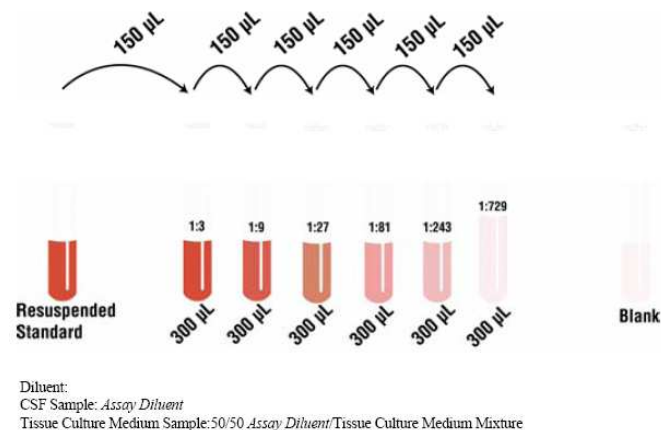
Standards may be analyzed alone, or may be combined with other protein standards for higher levels of multiplexing.

DO NOT COMBINE MORE THAN 4 STANDARDS.

☞ Please see the **INFORMATION SHEET** included with the Antibody Bead Kit(s) used for details.

### ● Prepare the Standard Curve

The standard curve is made by serially diluting the reconstituted standard in *Assay Diluent* (CSF samples) or 50% *Assay Diluent*/50% culture medium (tissue culture medium samples). See diagram below. Do not vortex. Mix by pipetting up and down 5-10 times.



Discard all remaining reconstituted and diluted standards after completing the assay. Return the *Assay Diluent* to the refrigerator.

## PREPARATION OF ANTIBODY CONJUGATED BEADS

### ● Bead Preparation for the FIDIS™ Human Neuroscience Cytokine Core Panel Assay

Determine the number of wells required for the assay. Standard curves and samples may be run singly or in replicates, as desired.

The beads are provided as a 10x concentrate. Immediately before dispensing, vortex the 10x bead concentrate for 30 seconds, then sonicate for 30 seconds. To make a 1x capture bead stock for the **FIDIS™ Human Neuroscience Cytokine Core Panel Assay**, mix 1 part 10x bead concentrate with 9 parts Working Wash Solution. Each well requires 25 µL of the diluted beads. ☞ See table below for dilution recommendations.

Diluted beads may be stored overnight at 2 to 8°C.

Number of Wells	Vol. 10x Beads	Vol. Working Wash Solution	Total Vol.
16	0.05 mL	0.450 mL	0.5 mL
24	0.07 mL	0.630 mL	0.7 mL
32	0.09 mL	0.810 mL	0.9 mL
40	0.11 mL	0.990 mL	1.1 mL
48	0.13 mL	1.17 mL	1.3 mL
56	0.15 mL	1.35 mL	1.5 mL
64	0.17 mL	1.53 mL	1.7 mL
72	0.19 mL	1.71 mL	1.9 mL
80	0.21 mL	1.89 mL	2.1 mL
88	0.23 mL	2.07 mL	2.3 mL
96	0.25 mL	2.25 mL	2.5 mL

**NOTE:** We include 4 extra volumes in our calculations to assure enough reagent when pipetting.

### ● Bead Preparation for a Multiplexed Assay

The bead component of the Neuroscience Cytokine Core Panel may be mixed with the bead components of other Neuroscience Antibody Bead Kits for higher level multiplexing. Please see Appendix I for details.

## PREPARATION OF DETECTOR ANTIBODY

### ● Detector Antibody Preparation for the FIDIS™ Human Neuroscience Cytokine Core Panel Assay

The Detector Antibodies are provided as 10x concentrates. To make a 1x Detector Antibody stock for the Neuroscience **FIDIS™ Human Neuroscience Cytokine Core Panel Assay**, mix 1 part 10x Detector Antibody Concentrate with 9 parts Detector Antibody Diluent. Each well requires 50 µL of the diluted Detector Antibody. See table below for dilution recommendations. Diluted Detector Antibody may be stored overnight at 2 to 8°C.

Number of Wells	Vol. 10x Beads	Vol. Working Wash Solution	Total Vol.
16	0.100 mL	0.90 mL	1.0 mL
24	0.140 mL	1.26 mL	1.4 mL
32	0.180 mL	1.62 mL	1.8 mL
40	0.220 mL	1.98 mL	2.2 mL
48	0.260 mL	2.34 mL	2.6 mL
56	0.300 mL	2.70 mL	3.0 mL
64	0.340 mL	3.06 mL	3.4 mL
72	0.380 mL	3.42 mL	3.8 mL
80	0.420 mL	3.78 mL	4.2 mL
88	0.460 mL	4.14 mL	4.6 mL
96	0.500 mL	4.50 mL	5.0 mL

**NOTE:** We include 4 extra volumes in our calculations to assure enough reagent when pipetting.

### ● Detector Antibody Preparation for a Multiplexed Assay

The bead component of the Neuroscience Cytokine Core Panel may be mixed with the bead components of other Neuroscience Antibody Bead Kits for higher level multiplexing. Please see Appendix I for details.

## ASSAY PROCEDURE

1. Pre-wet the wells designated for the assay. Pipette 0.2 mL of Working Wash Solution into designated wells. Wait 15 to 30 seconds then aspirate the Working Wash Solution from the wells using the vacuum manifold. Wells not used during the assay should be kept dry for future use. An adhesive plate cover may be used to seal the unused wells.
2. Vortex the diluted bead solution for 30 seconds, then sonicate for at least 30 seconds immediately prior to use in the assay.
3. Pipette 25 µL of the diluted bead solution into each well. Once the beads are added to the plate, keep the plate shielded from light.
4. Add 0.2 mL Working Wash Solution to the wells.

Allow the beads to soak for 15 to 30 seconds, then remove the Working Wash Solution from the wells by aspiration with the vacuum manifold. Solution from the wells by aspiration with the vacuum manifold. Repeat this washing step. Blot the bottom of the filter plate on clean paper towels to remove residual liquid.

5. Pipette 50  $\mu$ L 1x Detector Antibody into all wells.
6. Pipette 50  $\mu$ L standards into designated wells.
7. Pipette 25  $\mu$ L *Assay Diluent* followed by 25  $\mu$ L samples into designated wells.
8. Incubate the plate for 3 hours, in the dark or covered, at room temperature on an orbital shaker. Shaking should be sufficient to keep beads suspended during the incubation (500-600 rpm). Overnight incubation is not recommended.
9. Ten to fifteen minutes prior to the end of this incubation, prepare the RPE conjugate. The conjugate is provided as a 10x concentrate. Dilute 1 part *RPE Conjugate 10x Concentrate* with 9 parts *RPE Diluent*. Each well requires 100  $\mu$ L of prepared 1x RPE Conjugate. The RPE Conjugate must be prepared fresh for each assay and be protected from light. See table below for dilution recommendations.

**RPE Conjugate Preparation for Single Analyte and Multiplexed Assays**

Number of Wells	Vol. 10x Beads	Vol. Working Wash Solution	Total Vol.
16	0.200 mL	1.8 mL	1.0 mL
24	0.280 mL	2.52 mL	1.4 mL
32	0.360 mL	3.24 mL	3.6 mL
40	0.440 mL	3.96 mL	4.4 mL
48	0.520 mL	4.68 mL	5.2 mL
56	0.600 mL	5.40 mL	6.0 mL
64	0.680 mL	6.12 mL	6.8 mL
72	0.760 mL	6.84 mL	7.6 mL
80	0.840 mL	7.56 mL	8.4 mL
88	0.920 mL	8.28 mL	9.2 mL
96	1.00 mL	9.00 mL	10.0 mL

**NOTE:** We recommend preparing reagent for 4 extra wells to assure sufficient volume for pipetting.

10. After the 3 hour incubation, remove the liquid from the wells by aspiration with the vacuum manifold. Add 0.2 mL Working Wash Solution to the wells. Allow the beads to soak for 15 to 30 seconds, then aspirate with the vacuum manifold. Repeat this washing step three more times for a total of four washes. Blot the bottom of the filter plate on clean paper towels to remove residual liquid.
11. Add 100  $\mu$ L of prepared 1x RPE Conjugate to each well.
12. Incubate the plate for 30 minutes, in the dark or covered, at room temperature on an orbital shaker. Shaking should be sufficient to keep beads suspended during the incubation (500-600 rpm).
13. Prepare the Luminex 100™ instrument during this incubation step. Please see **APPENDIX II**.
14. After the 30 minute incubation, remove the liquid from the wells by aspiration with the vacuum manifold. Add 0.2 mL Working Wash Solution to the wells. Allow the beads to soak for 15 to 30 seconds, then aspirate with the vacuum manifold. Repeat this washing step three more times for a total of four washes. Blot the bottom of the filter plate on clean paper towels to remove residual liquid.
15. Add 100  $\mu$ L of Working Wash Solution to each well. Shake the plates on an orbital shaker (500-600 rpm) for 2 to 3 minutes to resuspend the beads. Uncover the plate; insert the plate into the XY platform of the FIDIS™ instrument, and analyze the samples.
16. Determine the concentration of samples from the standard curve using curve fitting software. The four parameter algorithm usually provides the best fit. **IMPORTANT:** In addition to dilutions performed on the sample prior to running the assay, the sample concentration calculated from the standard curve must be multiplied by an additional factor of 2 to correct for the 1:2 dilution in Step 7.

## LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the highest standard point; the dose-response is non-linear in this region and inaccurate. Dilute samples that are greater than the highest standard with *Assay Diluent* or appropriate matrix diluent; reanalyze these samples and multiply results by the appropriate dilution factor. The influence of various drugs and the use of biological fluids in place of CSF and tissue culture medium samples have not been thoroughly investigated. The rate of degradation of analytes in various matrices may not have been investigated. The immunoassay literature contains frequent references to aberrant signals, attributed to heterophilic antibodies.

**This kit is for research use only.  
Not for human therapeutic or diagnostic use.**

## PERFORMANCE CHARACTERISTICS

Please refer to analyte specific **INFORMATION SHEET** for performance claims.

### APPENDIX I: MULTIPLEX ASSAY DEVELOPMENT

**FIDIS™ Human Neuroscience Cytokine Core Panel Kits** are designed to permit maximal flexibility in experimental design. These kits can be used alone, or combined with other **FIDIS™ Human Neuroscience Antibody Bead Kits** to enable the development of higher level multiplexed assays designed by the researcher.

In this Appendix, we present instructions for combining bead mixtures and Detector Antibody mixtures in the development of multiplexed assays.

Before preparing multiplexed assays, it is important to verify that each analyte is represented by a unique bead region. This assures the compatibility of each Neuroscience Antibody Bead Kit in the

development of multiplexed assays.

Up to 10 bead concentrates (pre-mixed multiplexes or single plexes) can be combined to increase the number of analytes being monitored.

### MULTIPLEXING CAPTURE BEADS

Each well requires 25  $\mu$ L of prepared 1x antibody conjugated beads. Instructions for preparing 1x beads for the **FIDIS™ Human Neuroscience Cytokine Core Panel** are presented on page 15 of this protocol. Beads from other **FIDIS™ Human Neuroscience Cytokine Kits** may also be combined with the **FIDIS™ Human Neuroscience Cytokine Core Panel beads** for higher levels of multiplexing. The volume of total 1x Bead Mixture, the volume of each 10x Bead Concentrate, and the volume of Working Wash Solution required for a multiplexed assay can be calculated by using the formulas presented below.

#### Total Volume of 1x Bead Mixture Required

$$(0.025 \text{ mL} \times (\# \text{ of wells} + 4)) = \underline{\hspace{2cm}}$$

#### Volume of each 10x Bead Concentrate Required

$$\text{Total Volume of 1x Bead Mixture Required} / 10 = \underline{\hspace{2cm}}$$

#### Volume Working Wash Solution Required

$$[\text{Total Volume of 1x Bead Mixture}] - [(\text{Volume 10x Beads}) \times (\# \text{ of plexes})] = \underline{\hspace{2cm}}$$

**To prepare a bead mixture for a multiplexed assay, pipette the beads and Working Wash Solution (using the volumes calculated with the formulas presented above) into a foil wrapped tube. Vortex the tube for 30 seconds, then sonicate for 30 seconds. The mixture is ready to be used in a multiplexed assay.**

If desired, premixed beads can be stored overnight at 2 to 8°C.

## MULTIPLEXING DETECTOR ANTIBODIES

Each well requires 50 µL of prepared 1x Detector Antibody.

Instructions for preparing 1x Detector Antibody for the Neuroscience Cytokine Core Panel are presented on page 16 of this protocol. Detector Antibody from other Neuroscience Antibody Beads Kits may also be combined with the Neuroscience Cytokine Core Panel Detector Antibody for higher levels of multiplexing. The volume of total 1x Detector Antibody, the volume of each 10x Detector Antibody Concentrate, and the volume of Detector Antibody Diluent required for a multiplexed assay can be calculated by using the formulas presented below.

### Total Volume of 1x Detector Antibody Mixture Required

$$(0.050 \text{ mL} \times (\# \text{ of wells} + 4)) = \underline{\hspace{2cm}}$$

### Volume of each 10x Detector Antibody Concentrate Required

$$\text{Total Volume of 1x Detector Antibody Mixture Required} / 10 = \underline{\hspace{2cm}}$$

### Volume Detector Antibody Diluent Required

$$[\text{Total Volume}] - [(\text{Volume 10x beads}) \times (\# \text{ of plexes})] = \underline{\hspace{2cm}}$$

To prepare a Detector Antibody mixture for a multiplexed assay, pipette the Detector Antibody Concentrates and Detector Antibody Diluent (using the volumes calculated with the formulas presented above) into a tube. Mix gently. The mixture is ready to be used in a multiplexed assay.

If desired, premixed 1x Detector Antibody can be stored overnight at 2 to 8°C.

## APPENDIX II: INSTRUMENT SETUP

### Helpful guides for FIDIS™ cytokine users

- Creating a Cytokine method, *April 2006*
- How to run Cytokine, *April 2006*

## REFERENCES

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**PROTOCOL SUMMARY**

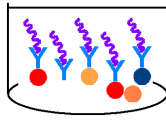
**Pre-wet plate**  
**Add 25µL 1x Antibody Coated Beads**  
**200µL Wash Solution**

↓ Wash 2 x 200µL

**Sample type:**

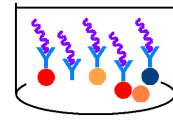
Standard

CSF/Tissue Culture Supernatant



Add 50µL  
Detection Antibody

Add 50µL  
Standard



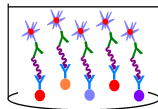
Add 50µL  
Detection Antibody

Add 25µL  
Assay Diluent

Add 25µL  
Sample



↓ Shake for 3 hours at R.T in the dark  
Wash 4 x 200µL



**Add 100µL 1 RPE**

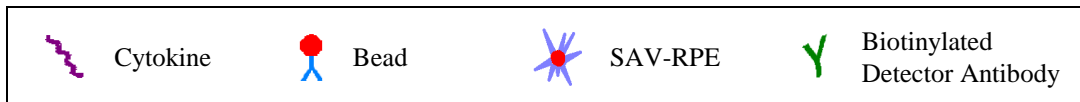
↓ Shake for 30 min. at RT in the dark  
Wash 4 x 200µL

**Add 100µL Wash Buffer**

↓ Shake for 2-3 min

**Read in FIDIS™ system**

**Total time: 3.5 hours**



**BioMédical Diagnostics SA**

**Office**

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