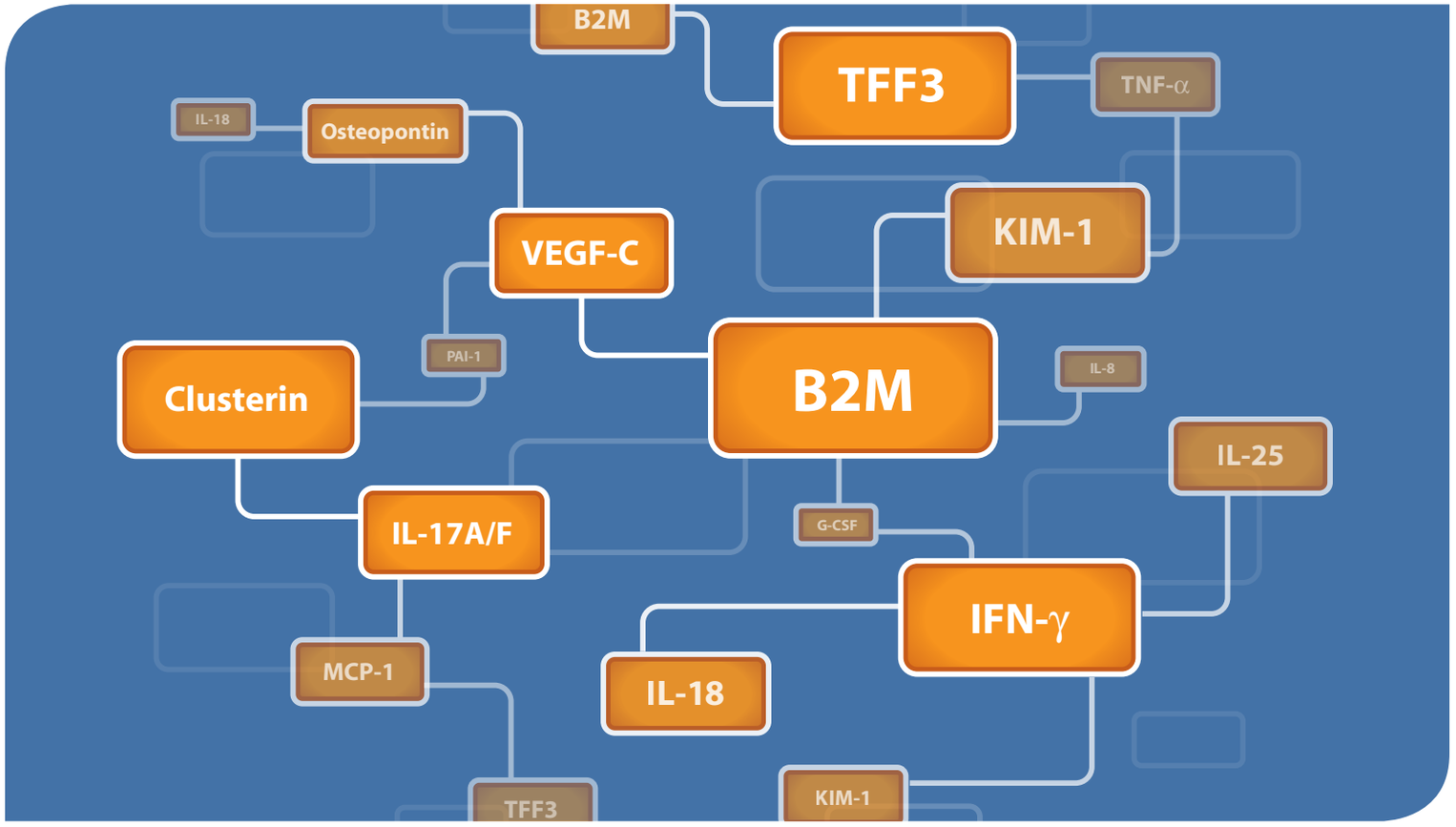

The Bio-Plex® Success Guide for Multiplex Immunoassays

Using Luminex xMAP Technology

Assay/Sample Prep

BIO-RAD

May the Plex Be with You

Episode I: I sense a disturbance with your assay/sample prep

Not too long ago, in a laboratory not too far away…

Luminex's xMAP technology was introduced to make your experiments faster and more efficient.

This is a success guide that includes a variety of tips and tricks for obtaining optimal results from your Luminex multiplex immunoassays.

(Always follow the product instruction manual when running an assay.)

Serum or Plasma: What Sample Do I Choose?

Sample measurements can differ for serum and plasma. Why?

- Serum is qualitatively different from plasma because of how the removal of fibrinogen and other proteins that form fibrin clots affects coagulation
- Protein concentration of serum is ~3–4% less than that of plasma

Choose sample matrix measurements that fall within the linear portion of the standard curve. This will allow for better precision and accuracy.

Avoid serum with gross lipemia or hemolysis.

Steps to a Successful Serum Prep:

1. Allow blood to clot at room temperature for 30–45 min before proceeding to centrifugation steps.
2. Centrifuge at 1,000 x g for 15 min at 4°C and transfer into a clean tube.
3. Repeat centrifugation at 10,000 x g for 10 min at 4°C to completely remove platelets and precipitates.
4. Dilute samples according to the assay instruction manual.

Aliquot and store undiluted sample at –80°C if not using immediately. Do not freeze diluted sample.

Serum separating tubes (SST) are preferred for superior separation quality.

Steps to a Successful Plasma Prep:

1. Centrifuge at 1,000 x g for 15 min at 4°C and transfer into a clean tube.
2. Repeat centrifugation at 10,000 x g for 10 min at 4°C to completely remove platelets and precipitates.
3. Dilute samples according to the assay instruction manual.

Use collection tubes coated with anticoagulants (such as Na citrate or EDTA) for better results.

Use protease inhibitor when profiling for diabetes/metabolic markers.

Note: You may have to centrifuge plasma again at 3,000 x g for 5 min if the sample clotted during extended storage. Care should be taken not to disturb pelleted material.

Steps to a Successful Cell Culture Supernatant Prep:

1. Centrifuge at 1,000 x g for 15 min at 4°C.

2. Supplement with serum (5–10%) or BSA (0.5–1%) to reconstitute the standard if using a serum-free culture supernatant.

3. Typically, cell culture supernatant is analyzed undiluted. However, if it is necessary to dilute the sample so that it is in the working range of the standard curve, ensure the sample contains the same amount of serum or BSA as the standard.

4. Aliquot and store at –80°C if not using sample immediately.

Steps to a Successful Cell and Tissue Homogenates Prep:

1. Rinse the tissue sample with cell wash buffer.*
2. If necessary, cut the tissue into small pieces (for example, 3 x 3 mm) and transfer to a tissue grinder on ice.
3. Dilute lysate at least twofold (1:2) in sample diluent** + 0.5% BSA, to a final protein concentration of 200–900 µg/ml.

For analytes with high expression, a lysate protein concentration as low as 50 µg/ml may be sufficient.

Standards should be reconstituted in the same buffer composition as your sample.

Dilute lysates as much as possible to reduce the detergent concentration. This will result in optimum antibody binding during sample incubation period.

* Available in Bio-Plex Pro™ cell signaling reagent kit (catalog #171-304006M).

** Available in Bio-Plex Pro reagent kit with flat bottom plate (catalog #171-304070M) or included in all preconfigured kits.

General Sample Tips to Make Your Life Easier!

Plan your experiment to ensure you use consumables from a single lot.

Consistency is the key:

- Use low protein binding aliquot tubes to maintain consistent assay performance
- Dispense reagents using multichannel pipets
- Ensure you use a lab timer to improve time uniformity between incubation steps in the assay
- Use dilution tubes to prepare serial dilution
- Use reverse-pipetting technique to enhance liquid dispensing accuracy
- Incubate the reconstituted standards on ice for 30 min
- Resuspend beads thoroughly before removing desired volume

Prepare 25% excess when possible.

Always run your standards, blanks, and samples in duplicates or more.

Strepavidin-PE is light sensitive:

- Cover the plate with foil during shaking steps
- Store reagents protected from light

Use a 200–300 µl capacity pipet to remove beads from the stock tube to minimize volume loss. If necessary, perform the volume transfer in two steps.

If not using immediately, then make sure your sample is stored at –80°C in single-use aliquots and minimize the number of freeze/thaw cycles.

For more assistance, visit our support website at
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