Proper sample handling for immunoassays

Proper sample handling is critical before running a protein quantitation assay, whether it's an ELISA, Luminex™ multiplex assay, or any other type of immunoassay. Below is a set of best practices and guidelines for common sample types to help ensure that your samples are in the best condition possible before running your assay.

General best practices

- Maintain samples at 2–8°C while handling, but allow the samples to come to room temperature before running the assay
- Avoid multiple freeze/thaw cycles of sample
- Frozen samples should be allowed to thaw on ice just prior to running the assay
- Upon thawing, samples should be clarified by centrifugation at 14,000 rpm for 10 minutes at 4°C in a refrigerated microcentrifuge or filtered prior to analysis
- Follow the assay protocol provided with the kit for appropriate dilutions

Cell culture supernatant

1. Stimulate cells as desired in the appropriate cell culture flasks. Cells should be in log phase growth when harvesting supernatant.
2. Using sterile technique, remove the desired volume of conditioned cell culture medium with a pipette and transfer the medium to clean polypropylene microcentrifuge tubes.
3. Centrifuge the medium at 14,000 rpm for 10 minutes at 4°C in a refrigerated microcentrifuge to remove any cells or cellular debris.
4. Aliquot the clarified medium into clean polypropylene microcentrifuge tubes.
5. If supernatant is to be analyzed at a later date, dispense into aliquots and store at –80°C.

Serum

1. Collect blood samples in pyrogen- and endotoxin-free tubes.
2. Allow whole blood to sit at room temperature for 15–30 minutes to clot.
3. Spin at 1,000–2,000 x *g* for 10 minutes at 4°C in a refrigerated centrifuge to separate the cells.
4. Transfer the supernatant to a clean, chilled polypropylene tube with a sterile Pasteur pipette.
5. If serum is to be analyzed at a later date, dispense into aliquots in polypropylene microcentrifuge tubes and store at –80°C. When possible, avoid the use of hemolyzed or lipemic serum.

Plasma

1. Remove cells from plasma samples by centrifugation at 2,000 x *g* for 10 minutes at 4°C in a refrigerated centrifuge. Centrifugation at this force is necessary to deplete platelets from the sample.
2. Transfer the supernatant to a clean, chilled polypropylene tube with a sterile Pasteur pipette.
3. If the plasma is to be analyzed at a later date, dispense into aliquots in polypropylene microcentrifuge tubes and store at –80°C.

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Tissue homogenate

The following protocol was developed using Invitrogen™ Tissue Extraction Reagent I (Cat. No. FNN0071; formulation listed below) and shows good correlation between ELISA and Luminex assays. This procedure has been applied to multiple tissue types, but it is recommended that you optimize for each sample type used. Similar extraction reagents and lysis buffers may be used.

Extraction efficiency will vary from tissue to tissue, as well as from marker to marker, with different extraction buffer formulations. Some markers and tissue types may require very strong extraction buffers, while other markers and tissue types may only need mild extraction buffers. It is common for researchers to perform small pilot studies with 3 or 4 different extraction buffers to empirically identify the optimal extraction buffer suitable for the majority of the markers in their specific test system. Multiple dilutions of the tissue homogenate should also be examined to determine the optimal sample dilutions needed in a particular study.

A total protein assay is recommended to normalize samples to one concentration before running the immunoassay. Best practice is to keep cell count consistent between samples or keep track of cell number collected per tissue culture sample.

1. Add protease inhibitors to Tissue Extraction Reagent I just before use.
2. Weigh tissue sample.
3. Add 10 mL of Tissue Extraction Reagent I per 1 gram of tissue.
4. Homogenize the tissue (best practice is to use ice-cold buffers and homogenize tissue on ice).
5. Centrifuge the sample at 10,000 rpm for 5 minutes at 4°C to pellet the tissue debris.
6. Collect the supernatant. The sample is ready to be used.*
7. If the sample is to be stored, dispense into aliquots and freeze at –80°C.

Tissue Extraction Reagent I formulation

50 mM Tris, pH 7.4
250 mM NaCl
5 mM EDTA
2 mM Na_3VO_4
1 mM NaF
20 mM $Na_4P_2O_7$
0.02% NaN_3
Proprietary detergent

* To minimize potential inhibition of antibody-antigen binding by the detergent present in the extraction or lysis buffer, the tissue homogenate or cell lysate sample must be diluted at least 5–10 fold (depending on the lysis buffer used) to reduce the detergent concentration to less than or equal to 0.01%. However, individual kits or samples may require further dilution in assay diluent or standard diluent buffer.

Ordering information

Product	Quantity	Cat. No.
Cell or tissue extraction buffer**		
Cell Extraction Buffer	100 mL	FNN0011
NP40 Lysis Buffer	100 mL	FNN0021
Tissue Extraction Reagent I	100 mL	FNN0071
Denaturing Cell Extraction Buffer	100 mL	FNN0091

** Cell or tissue extraction buffers are not included in any assay kits.

Find out more at **thermofisher.com/immunoassays**

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