RayBiotech, Inc
the protein array pioneer company

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GENERAL TIPS FOR SAMPLE PREPARATION

Note: In case follow-up experiments are needed, it is strongly recommended to sub-aliquot all samples after preparation to minimize cytokine degradation from multiple freeze-thaw cycles.

How do I prepare conditioned media samples?

We recommend preparing serum-free or low-serum medium samples, as serum tends to contain cytokines which may produce significant background signals. If it is necessary to test serum-containing medium, we recommend also running an uncultured media blank to assess baseline signals. This baseline can then be subtracted from the cultured media sample data.

1. On day 0, seed ~1 million cells in 100 mm tissue culture plate with complete medium.*
2. On day 3, remove medium and replace medium with 6-8 ml of serum-free or low serum containing medium (e.g. medium containing 0.2% calf serum).
3. On day 5, collect medium into 15 ml tube. Centrifuge at 2,000 rpm in centrifuge at 4ºC for 10 minutes. Save the supernatant. Transfer the supernatant into 1.5 ml Eppendorf tubes. Store supernatant at -80ºC until experiment. Most samples can be stored this way for at least a year.

**The optimal number of seeded cells varies from one cell type to another and may need to be empirically determined.*

How do I prepare plasma and serum samples?

For plasma:

1. Collect whole blood into an EDTA, Citrate or Sodium heparin tube (e.g. BD vacutainer, Cat. No: 8001302 or 16852).
2. Centrifuge 10 minutes at 3,000 rpm
3. Aliquot into small tubes and store at -80^{0}C until use.

For serum:

1. Collect whole blood into a tube without additives (e.g. BD vacutainer, Cat. No. 8002527).
2. Keep at room temperature for 20 minutes.
3. Centrifuge 10 minutes at 3,000 rpm.
4. Aliquot into small tubes and store at -80^{0}C until use.

How do I prepare urine samples?

1. Collect urine without adding stabilizers.
2. Centrifuge the samples hard (eg. 10,000 x g for 1 min or 5,000 x g for 2 min).
3. Aliquot, quick freeze in dry ice/methanol bath, and store at -80^{0}C until use.

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How do I prepare cell or tissue lysate samples?

Cell or tissue lysates for use with RayBio® Antibody Arrays and ELISA kits can be prepared using most conventional methods, e.g. homogenization of cell or tissue in RayBio® Lysis Buffer. You may also use your own lysis buffer, such as RIPA or other formulations optimized for immunoprecipitation. Please note the following guidelines on lysis buffer composition:

1) Avoid using >0.1% SDS or other strongly denaturing detergents. In general, non-ionic detergents such as Triton X-100 or NP-40 are best, although zwitterionic detergents such as CHAPS, or mild ionic detergents such as sodium deoxycholate will work.
2) Use no more than 2% v/v total detergent
3) Avoid the use of sodium azide
4) Avoid using >10 mM reducing agents, such as dithiothreitol or mercaptoethanols

We strongly recommend adding a protease inhibitor cocktail to the lysis buffer prior to homogenization. Most general biochemical supply companies including Roche, Sigma-Aldrich, Pierce, and Calbiochem stock a wide variety of these products. Since susceptibility to proteolytic cleavage and the type of proteases present in the lysate vary, we do not recommend a specific product. Instead, your choice of which combination of protease inhibitors to use should be based upon a literature search for your protein(s) of interest and/or tissue or cell type. Phosphatase inhibitors may be used but are not necessary unless the antibodies used in the kit specifically recognize phosphorylated forms of the protein.

Choices of the method for lysis and homogenization include glass-bead “smash,” douncing, freeze-thaw, sonication and crushing frozen tissue with a mortar and pestle, or even a combination of these. There is no best method for all sample types; your choice of method should be made following a brief search of the literature to see how samples similar to yours have been prepared in previous investigations.

After homogenization, centrifuge the lysates to remove cell/tissue debris (5 min @ 10,000 x g or 10 min @ 5,000 x g) and save the supernatant. Unless testing fresh, lysates should be frozen as soon as possible and stored at -20°C (or -80°C, if possible). Centrifuge them again before incubating with any immunoassay. Next, determine the protein concentration of your lysates using a total protein assay not inhibited by detergents (such as the Bicinchoninic acid (BCA) assay) and normalize the volume of each sample used to deliver the same amount of total protein for each assay.

Note: The Bradford assay is not recommended as it can be inhibited by the presence of detergents.

Since different cells and tissues may contain different amounts of protein, as starting point, we suggest using 500 µL of lysis buffer per $1x10^6$ cells or 10 mg tissue. You may have to adjust this based upon your results. Your target total protein concentration of the homogenate should be at least 1,000 µg/mL, but 2,000 µg/mL or more would be better.