Now Includes Protocol for Bio-Plex Phospho-Histone H3 Lysate Peparation!

Bio-Plex™ Cell Lysis Kit Product Insert

For use with
Bio-Plex phosphoprotein assays and
Bio-Plex total target assays

For research use only.
Not for diagnostic procedures.

BIO-RAD

For technical service, call your local Bio-Rad office,
or in the US, call 1-800-4BIORAD (1-800-424-6723)

4110051 Rev D

Introduction

The Bio-Plex cell lysis kit has been developed specifically to prepare cell culture and tissue lysate samples for analysis with Bio-Plex phosphoprotein and total target assays. The cell lysates can be tested for the presence of phosphorylated proteins using Bio-Plex phosphoprotein assays or for the abundance of target proteins using Bio-Plex total target assays. This cell lysis kit can also be used to prepare cell lysates for western blot analysis (request bulletin 3033).

Product Description

The following components are provided with the Bio-Plex cell lysis kit:
Cell wash buffer
Cell lysis buffer
Cell lysis buffer, factor 1 (250x)
Cell lysis buffer, factor 2 (500x)

Storage and Stability

The cell wash buffer and cell lysis buffer should be stored at 4ºC. Factors 1 and 2 should be stored at –20ºC and can be frozen and thawed up to 5 times. All components are guaranteed for 6 months from the date of purchase when stored as specified.

Materials Required but Not Supplied

Phenylmethylsulfonyl fluoride (PMSF),
Sigma catalog #P7626

Dimethyl sulfoxide (DMSO),
Sigma catalog #D2650

Laemmli sample buffer,
Bio-Rad catalog #161-0737

2-Mercaptoethanol,
Bio-Rad catalog #161-0710

Lysate Preparation

Adherent and Suspension Cell Preparation

1. Rinse the samples with *cell wash buffer* as follows:

   Adherent Cells — Stop the treatment reaction by aspirating the culture medium and quickly rinsing the cells with ice-cold cell wash buffer. The volume of cell wash buffer required is the same as the volume of aspirated cell culture medium. Keep the cells on ice.

   Suspension Cells — Stop the treatment reaction by adding ice-cold wash buffer to the cells. The volume of cell wash buffer required is twice that of the culture medium. Centrifuge the cells at 1,000 rpm for 5 min at 4ºC. Aspirate the supernatant.

   Tissue Samples — Rinse the tissue sample with cell wash buffer once. Cut the tissue into 3 x 3 mm pieces and transfer them to a 2 ml tissue grinder.

2. Prepare 500 mM PMSF by dissolving 0.436 g PMSF in 5 ml DMSO. Store as 0.5 ml aliquots at –20ºC. Aliquots can be frozen and thawed up to 5 times.

3. Prepare an adequate volume of lysing solution (refer to the table on the left). For 10 ml of lysing solution, add 40 µl of *factor 1* and 20 µl of *factor 2* to 9.9 ml of *cell lysis buffer*. Vortex gently to mix and set aside on ice. Then add 40 µl of 500 mM PMSF.

Lysing Solution Volume Guide

Culture vessel	Culture medium volume	Lysing solution volume	Notes
96-well plate	100 µl/well	75 µl/well	Grow cells to 80–85% confluence Recommend leaving external wells empty due to edge effect
10 cm culture dish	10 ml	2–3 ml	Grow cells to 80–90% confluence

4. Lyse the samples:

Adherent and Suspension Cells

a) Immediately add the lysing solution to the cells. The amount of lysing solution needed depends on the cell concentration in the culture vessel (see table on the left).

b) Agitate the cells as follows:

Culture Plate — For suspension cells, place the plate on ice and pipet the contents of the wells up and down 5 times. For adherent cells, scrape the cells with a cell scraper. For both, agitate the plate on a microplate shaker at 300 rpm for 20 min at 4ºC.

Other Culture Vessel — Transfer the cell lysate to a centrifuge tube and rotate for 20 min at 4ºC.

HINT: Freeze-thawing the lysate once using dry ice or a –20ºC freezer may increase the extent of the lysis. Alternatively, briefly sonicate (eg., with a Sonifier 450 as follows: Duty cycle = 40, Output = 1, Pulse sonicating = 18 times).

c) Centrifuge the samples at 4,500 g for 20 min at 4ºC.

Tissue Samples

a) Immediately add 500 µl of lysing solution to the tissue grinder and grind the tissue sample on ice using about 20 strokes.

b) Transfer the ground tissue to a clean microcentrifuge tube and freeze the sample at –70ºC.

c) Thaw the samples, then sonicate on ice as suggested above.

d) Centrifuge the samples at 4,500 g for 4 min.

5. Collect the supernatant without disturbing the pellet.

Suggested Protocol for Lysate Preparation of Histone H3 Assay:

1. Follow steps 1-3 in lysate preparation.

4. Lyse the samples:

   a) Immediately add the lysing solution to the cells. The amount of lysing solution needed depends on the cell concentration in the culture vessel (see table on the left).

   b) Briefly sonicate (e.g., with a Sonifer 450 as follows: Duty cycle = 40, Output = 1, Pulse sonicating = two 10 minute pulses with a 1 minute break in between).

   c) Agitate the cells. Transfer the cell lysate to a centrifuge tube and rotate for 20 min at 4ºC.

   d) Centrifuge the samples at 4,500 g for 20 min at 4ºC.

5. Collect the supernatant without disturbing the pellet.

For Bio-Plex Phosphoprotein Assays and Bio-Plex Total Target Assays

1. Determine the lysate protein concentration. The protein concentration should be 200–900 µg/ml. It may be necessary to test-lyse your samples with different volumes of lysing solution to obtain the specified protein concentration range.

2. Add an equal volume of *assay buffer* to the lysate.

3. If the lysate is not tested immediately, store at –20ºC. The lysate is stable for up to 5 freeze-thaw cycles. For Bio-Plex Histone H3 assay, freeze lysate (overnight) at –20ºC and thaw before testing.

4. For further assay instructions refer to the Bio-Plex phosphoprotein detection instruction manual.

For Western Blot Analysis

1. Determine the lysate protein concentration. The protein concentration should be 200–900 µg/ml. It may be necessary to test-lyse your samples with different volumes of lysing solution to obtain the specified protein concentration range.

2. If the lysate is not tested immediately, store at –20ºC. The lysate is stable for up to 5 freeze-thaw cycles.

3. Prepare fresh sample loading buffer using a 1:20 dilution of 2-mercaptoethanol and Laemmli sample buffer. Alternatively, another sample loading buffer can be used.

4. Dilute 1 part sample with 2 parts sample loading buffer.

5. For further instructions, refer to Bio-Rad's Laemmli sample buffer manual.

Safety Considerations

Eye protection and gloves are recommended while using this product. Consult the MSDS for additional information.