

# MSD<sup>®</sup> Lysate Preparation Protocol

## General notes:

All manipulations should be performed on ice. Prepare desired amount of Complete Lysis Buffer. Lysis volumes will vary between cell types. Larger cells (such as NIH3T3, HeLa) should be lysed at concentrations of  $1-5 \times 10^6$  cells per mL of Lysis Buffer. Smaller cells (such as Jurkat) should be lysed at concentrations of  $1-5 \times 10^7$  cells per mL of Lysis Buffer.

## Reagents required:

### Lysis Buffer (1X)

150 mM NaCl  
20 mM Tris, pH 7.5  
1 mM EDTA  
1 mM EGTA  
1% Triton X-100

*10 mM NaF and phosphatase/protease inhibitors are added immediately prior to use*

### Complete Lysis Buffer

10 mL 1X Lysis Buffer  
1 Protease inhibitor tablet (Roche, Catalog #1 836 170)\*\*  
100  $\mu$ L Phosphatase inhibitor 1 (Sigma, Catalog # P-2850, 100X stock)  
100  $\mu$ L Phosphatase inhibitor 2 (Sigma, Catalog # P-5726, 100X stock)  
100 $\mu$ L NaF (from 1M stock)\*\*

\*\* In the reagents supplied by MSD, the protease inhibitor tablet and the NaF have been combined into a 50X protease inhibitor cocktail.

### **NOTE:**

*For assays that require PMSF, a final concentration of 2 mM in Complete Lysis Buffer is recommended.*

For 10 mL Complete Lysis Buffer, add 40  $\mu$ L PMSF (from 500 mM stock prepared in DMSO) (Sigma, Catalog # P-7626).

When adding PMSF to the buffer, all reagents should be at room temperature prior to mixing. The Complete Lysis Buffer should be mixed at room temperature on a rotator for 5 minutes (with no obvious precipitates), and should then be thoroughly chilled prior to use for lysate dilution or preparation.

*When analyzing cells for p38 alone, the following Lysis Buffer affords optimal performance:*

**p38 Lysis Buffer (1X)**

50 mM NaCl

20 mM Tris, pH 7.5

1 mM EDTA

1 mM EGTA

1% Triton X-10

Prepare Complete p38 Lysis Buffer as shown above, substituting the 10 mL of Lysis Buffer (1X) with p38 Lysis Buffer (1X).

## ***Protocol***

Cells should be prepared as desired to activate target protein.

### **Suspension cells**

Pellet cells (500 x g, 3 minutes at 4°C) and wash one time with cold 1X PBS. Pellet cells again and resuspend in 1X Complete Lysis Buffer at 1-5 x 10<sup>7</sup> cells per mL. Incubate on ice for 30 minutes (a shorter incubation time of 15 minutes may be adequate for many targets). Centrifuge lysates at ≥ 10,000 x g, 4°C for 10 minutes to clear cellular debris from the lysate.

Lysates can be quantitated using a detergent compatible protein assay such as BCA. Unused lysates should be aliquoted and snap frozen and stored at –80°C.

### **Adherent cells**

All volumes are determined for cells plated in 15 cm dishes. Remove media from the plates and wash cells one time with 5 mL cold 1 X PBS. Add 2 mL 1 X PBS to the plates and scrape the cells from the surface of the dish and transfer into 15 mL conical tubes. Pellet the cells at 500 x g for 3 minutes at 4°C. Resuspend the cells in 0.5-2 mL of Complete Lysis Buffer per dish. Alternatively after medium removal, cells can be washed one time with PBS including a careful aspiration of residual PBS and lysed directly on the dish by adding 1-2 mL (depending on cell type) of Complete Lysis Buffer per dish. Incubate on ice for 30 minutes (a shorter incubation time of 15 minutes may be adequate for many targets). Centrifuge lysates at ≥ 10,000 x g, 4°C for 10 minutes to clear cellular debris from the lysate.

Lysates can be quantitated using a detergent compatible protein assay such as BCA. Unused lysates should be aliquoted and snap frozen and stored at –80°C.

### **96 well format modifications**

Successful adaptation to a 96 well format is cell type and target-dependent. The number of cells to be plated per well should be determined per cell type. General recommended plating concentrations for adherent cells range from 1 x 10<sup>4</sup>-5 x 10<sup>4</sup> cells per well, and approximately 2 x 10<sup>6</sup> cells per mL (50-75 μL per well) for suspension cells. These numbers are provided as a guide and the optimal concentrations will vary depending upon cell line used.



### **Suspension cells**

For flat bottom plates, experiments should be designed such that the final volume per well is 50-75  $\mu\text{L}$ . Perform cell lysis using a 4X Complete Lysis Buffer concentrate, supplemented with protease and phosphatase inhibitors at 4X concentrations. Add 4X Complete Lysis Buffer directly to cells in the growth medium for a final 1X concentration in the well.

NOTE: With some effort, a 10X Complete Lysis Buffer can also be prepared. *(For conical microwell plates, perform lysis by pelleting the cells, removing most of the growth medium and adding a constant amount of 1X Complete Lysis Buffer.)*

### **Adherent cells**

Plate cells on biologically treated tissue culture ware (such as BIOCOAT™) to reduce variability due to cells lost as growth medium is removed. Treat cells as desired. Gently aspirate growth medium from microwell plate. A PBS wash step is not required and can introduce variability. Add 50-100  $\mu\text{L}$  1X Complete Lysis Buffer per well.

Cell lysis time should be determined by the end user. Some targets are immediately available for detection, while others may require an incubation step at room temperature, 4°C, or on ice with gentle agitation.

Carefully pipet cell lysate onto prepared capture plate. It is important to transfer a constant volume and avoid pipeting too vigorously, as the introduction of air bubbles may result. *(Targets can be captured from a volume greater than 25  $\mu\text{L}$ .)*