



## Whole Cell Extraction Reagent for *Transcription Factor Analysis*

Store at 4°C

**Catalog No. 11941-100 100 Reactions**

### Overview and Intended Use

The Marligen Whole Cell Extraction Reagent provides material for preparing whole cell extracts from 100 samples having a cell pellet volume of 50 $\mu$ L. The protocol can be scaled to accommodate samples comprising larger or smaller pellet volumes and the number of reactions will vary accordingly.

### Principle of Method

Cultured cells are collected by centrifugation and washed in phosphate buffered saline to remove cell culture medium. Cells are then lysed in Complete Whole Cell Lysis Buffer. The whole cell extract is clarified by centrifugation, and stored at -80°C until use.

### Important Information

#### READ ENTIRE PROTOCOL BEFORE USE

This procedure must be carried out using cold reagents and tubes. Maintain reagents and tubes on ice during procedure.

### Safety and Use Statement

All biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of potentially infectious or hazardous agents.

This product is authorized for laboratory research use only. The product has not been qualified or found safe and effective for any human or animal diagnostic application. Uses other than the labeled intended use may be a violation of applicable law.

### Components included with this kit:

Whole Cell Extraction Reagent (10mL)

### Materials and equipment required but not supplied:

Phosphatase Inhibitor cocktail (Sigma Catalogue no. P5726)

Protease Inhibitor cocktail (Sigma Catalogue no. P8340)

0.1 M DTT (dithiothreitol, Sigma Catalogue no. D9779) in deionized water (store @ -20°C)

100 mM PMSF (Sigma P7626) in 100% ethanol (store @ -20°C)

**Cold** Phosphate Buffered Saline, pH 7.4

**Cold** 5mL, 15mL, or 50mL conical tubes

**Cold** Microcentrifuge tubes

Refrigerated microcentrifuge

### Additional material required for adherent cells:

**Cold** Phosphate Buffered Saline without Calcium or Magnesium, pH 7.4

**Cold** Phosphate Buffered Saline with 5mM Sodium EDTA, pH 7.4 (without Calcium or Magnesium)

### Set Up

Determine the number of tubes required for the number of samples being prepared and chill by placing on ice for at least 15 minutes prior to use.

Remove reagents from refrigeration and place on ice. Keep ALL reagents on ice throughout this procedure.

Calculate the approximate quantity of each reagent required for the Complete Whole Cell Lysis Buffer (see step 7).

Prechill an appropriate container for each reagent by placing on ice. Thaw protease inhibitors, DTT, and PMSF on bench top.

**NOTE: DO NOT PREPARE COMPLETE WHOLE CELL LYSIS BUFFER AT THIS TIME. THIS SHOULD BE PREPARED IMMEDIATELY PRIOR TO USE.**

### Protocol for Suspension Cells

NOTE: All steps should be performed on ice or in a refrigerated centrifuge.

1. Determine cell density in liquid media.
2. Aliquot up to 2 x 10<sup>7</sup> cells per tube into 15 mL conical tubes.

3. Collect the cells by centrifugation at 800 x g for 5 minutes at 4°C. Remove supernatant.
4. Add 10 mL cold PBS, resuspend cells and pellet by centrifugation. Remove supernatant. Repeat for a total of 2 PBS washes.
5. Resuspend pellet in 1mL cold PBS by pipeting up and down and pellet cells by centrifugation 800 X g for 5 minutes in a refrigerated centrifuge.
6. Remove the supernatant slowly. Do not disturb the pellet. Discard the supernatant. Place the tube containing the cell pellet on ice. Estimate the volume of each pellet.
7. Immediately prior to use, prepare Complete Whole Cell Lysis Buffer in the prechilled container according to the chart below. Two (2) pellet volumes of Complete Whole Cell Lysis Buffer will be used for each sample. Volumes can be directly scaled to accommodate larger pellet volumes based on the requirement of two pellet volumes of Complete Whole Cell Lysis Buffer for each pellet.

Complete Whole Cell Lysis Buffer	Quantity needed for 1000 µl
Whole Cell Extraction Reagent	1000 µl
Protease Inhibitor	10 µl
Phosphatase Inhibitor	10 µl
0.1 M DTT	10 µl
0.1 M PMSF	1 µl

**NOTE: The Complete Whole Cell Lysis Buffer should be used within 10 minutes of preparation.**

8. Add 2 pellet volumes of Complete Whole Cell Lysis Buffer to each cell pellet. Resuspend each pellet by pipeting up and down and transfer to clean prechilled 1.5 mL microcentrifuge tubes. Incubate on ice for 10 minutes and then vortex for 5 seconds.
9. Centrifuge at high speed for 30 minutes in a refrigerated microcentrifuge.
10. Remove and save supernatant in a chilled tube. **This is the whole cell extract.**
11. Quantitate extracts prior to freezing. (The BCA Protein Assay (Pierce) is the recommended method). Dilute the extracts to the desired concentration (5- 10 µg/µL is recommended) in Complete Whole Cell Lysis Buffer.
12. Store extracts at –70°C to –80°C.

## Protocol for Adherent Cells

**Note: Keep cells on ice or refrigerated for this entire procedure.**

1. Remove cell culture media by aspiration and wash cells twice with an equal volume of ice- cold PBS (containing no calcium or magnesium).
2. Add an equal volume of ice-cold 5mM EDTA in PBS (containing no calcium or magnesium) to the cells and incubate on ice for 10 minutes.
3. Collect cells with a rubber policeman and transfer to a 15 mL conical tube. Continue with Step 3 of the **Protocol for Suspension Cells**.