10-min. Histone/DNA Binding Protein Extraction Kit (Cells)

Cat. #: P514-4 (4 reactions); P514-25 (25 reactions); P514-50 (50 reactions)

Storage: at room temperature **Shelf Life:** 12 months

Product Description

This kit is for high efficient extraction for both histones and other DNA binding proteins (regardless of binding affinity) from cultured cells or cells isolated from animal tissues, in less than 10 minute.

The extracted protein can be used for SDS-PAGE, immunoblotting, ELISA, IP, and protein localization and modification studies. This product is for research use only.

Product Components (Store at room temperature)

Components	Amount		
	Cat. #: **P514-4**	Cat. #: **P514-25**	Cat. #: **P514-50**
Buffer A	2 ml	12 ml	25 ml
Buffer B	1.5 ml	12 ml	25 ml
Protein extraction cartridges	4	25	50
Collection tubes with cap	4	25	50

Important note:

The use of protease inhibitors is optional for this kit. If downstream application takes significant amounts of time or the protein extract will be stored for longer period of time, addition of protease inhibitor to the extract is recommended.

For determination of protein concentration, BCA kit is recommended.

To study protein phosphorylation, phosphatase inhibitors (such as PhosStop from Roche) should be added to Buffer A prior to use.

**If precipitate is found in Buffer B at lower temperature, incubate at >37°C until the precipitate is completely dissolved

Additional Materials Required:

1 X PBS, Vortex, Table-Top Microcentrifuge, BCA Protein Assay Kit

Protocol:

1. Prior to performing the procedure, pre-chill filter cartridge(s) with collection tube(s) and Buffer A on ice.

   Collect 0.5-5 million cultured cells or cells isolated from tissues by low speed centrifugation, **500-600x g for 5 min.**

   If start with animal tissues, we recommend to use our **10-min. Tissue (Tumor) Dissociation / Single Cell Isolation Kit** (Cat.# P712, http://www.101bio.com/quick_order.php) to dissociate the tissue and obtain the single cell suspension.

2. Resuspend cell pellet in **1 ml cold PBS** and transfer the cell suspension to a 2.0 ml microcentrifuge tube. Centrifuge the tube at **3,000 rpm for 2 min** to pellet the cells. Remove supernatant completely.

3. Resuspend the cell pellet in **0.5 ml Buffer A** and incubate **on ice for 5 min**. Vortex the tube briefly and centrifuge at **14,000 rpm for 2 min**. Remove supernatant (containing cytosolic proteins) completely.

   Optional: Wash the pellet with 1.0 ml cold PBS.

4. Add proper amount of **Buffer B** (Table1 below, based on wet cell pellet volume or starting cell#) to the tube and **vortex vigorously for 10 seconds**.

   Immediately pour the content into **pre-chilled filter cartridge**, cap it and centrifuge at **14,000 rpm for 30 seconds**. Discard the filter cartridge according to your institution's waste disposal protocol.

   The flow through in collection tube contains extracted histone and DNA binding proteins (typically 1-2.5 mg/ml). If the extract is used for immunoprecipitation, dilute 1:3 with PBS-Tween or other suitable buffers.

Table 1, Buffer B volume for different number of starting Cells

Volume of cell pellet (µL)	Corresponding Cell# (Millions)	Buffer B volume (µL)
5	0.5	25 ~ 50
10	1.0	50 ~ 100
20	2.0	100 ~ 150
50	5.0	250 ~ 300

Troubleshooting

Problem	Solution
Low protein concentration	Increase amounts of starting cells or decrease amount of Buffer B
Retention of liquid in the filter	Reduce starting cell# or increase Buffer B

Remarks: This protocol is developed and validated by 101Bio's OEM partner. Spin column based protein extraction and cell. fractionation technologies were developed by 101Bio's OEM partner.

-- The end --