

Minute™ Histone/DNA Binding Protein Extraction Kit

Catalog number: HP-014

Description

Histones and other DNA binding proteins play an important role in chromosome organization, gene regulation and posttranslational modification. Core components of histones can be covalently modified through methylation, acetylation, phosphorylation, and sumoylation. These modifications are essential for gene expression, regulation, DNA repair and chromosome condensation. HP-014 is designed to rapidly extract histones and other DNA binding proteins from cultured cells and cells isolated from animal tissues. Unlike many other commercial histone extraction kits that employ acid or high salt extraction methods, HP-014 extracts histone proteins through a completely different mechanism. This kit is equally efficient in extracting histones and other DNA binding proteins that can be used for internal controls for histone modification analysis. In comparison, acid extraction protocol selectively extracts basic proteins and a good internal control is usually not possible. HP-014 can extract histones from 0.5 to 5 million cells in less than 10 min with high protein yield (1-2.5 mg/ml) making it the fastest and most robust histone extraction kit available in the market.

Application

The kit is designed to rapidly and efficiently extract histones and other DNA binding proteins (regardless of binding affinity) for applications such as SDS-PAGE, immunoblottings, ELISA, IP and protein localization and modification studies.

Buffer Formulation: Proprietary

Kit Components

1. 25 ml buffer A
2. 25 ml buffer B
3. 50 protein extraction cartridges
4. 50 collection tubes with cap

Shipping: This kit is shipped at ambient temperature

Storage: Store the kit at room temperature

Important Product Information

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 (Pierce) 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
 Vortexer
 Table-Top Microcentrifuge
 BCA Protein Assay Kit (Pierce, Cat # :23227)

Histone and DNA Binding Protein Extraction Procedures

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 (500-600 X g 5 min). Minute™ single cell isolation kit (Cat# SC-012, Invent Biotechnologies, MN) is recommended for obtaining single cell suspension from animal tissues.
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 3000 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 (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

| Wet Volume (µl) of cell pellet | Corresponding Cell# (Millions) | Buffer B ((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 |