Animal Cultured Cells and Tissues

Total Protein Extraction Kit for WB

Description:

Total protein extraction kit for animal cultured cells and tissues is the most advanced next generation protein extraction tool for super-fast protein extraction .More and more evidences have shown that the most commonly used RIPA buffer can cause unpredictable protein loss, resulting in questionable data interpretation. This problem is resolved by the spin column based technologies. Coupled with much stronger lysis buffers, proteins can be extracted more efficiently. Due to the use of the proprietary protein extraction filter cartridges, the extraction volume can be as low as 20 µl – a very useful feature in situations where starting material is a limiting factor.

Application:

Total protein extraction kit is designed to rapidly extract total proteins from invertebrate and vertebrate cultured cells and tissues for applications such as SDS-PAGE, immunoblotting.

Kit components:

1. 25 ml denaturing cell lysis buffer
2. 50 protein extraction filter cartridges
3. 50 collection tubes with cap
4. 2 Plastic rods

**NOTE: Cell lysis buffers listed above do not contain any reducing agents and primary amine

Shipping: This kit is shipped at ambient temperature

Storage: Store the kit at room temperature

Important Product Information

The total protein extraction kits are designed to extract total protein rapidly. The use of protease inhibitors is not necessary prior to extraction. However, if downstream application takes significant amounts of time or the protein extract will be stored for longer period of time, addition of protease inhibitors to cell lysis buffer is recommended. For determination of protein concentration, BCA kit is recommended. To study protein phosphorylation, phosphatase inhibitors should be added to lysis buffer prior to use.

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Additional Materials Required

1 X PBS

Vortexer

Table-Top Microcentrifuge

BCA Protein Assay Kit

Protocols:

Total Protein Extraction for Cultured Cells

A. Non-Adherent Cells

1. Prior to protein extraction, pre-chill the protein extraction filter cartridge with collection tube on ice.
2. Harvest cells by low speed centrifugation. Wash the cells in cold PBS once in a 1.5 ml microcentrifuge tube and pellet the cells by centrifugation at 500 X g for 2-3 min. Aspirate the supernatant and leave small amount of PBS (about the volume of packed cells) in the tube. Vortex the tube briefly to resuspend the cells.
3. Add appropriate amounts of cell lysis buffer to the cell suspension (Table 1), vortex briefly to lyse the cells.

Important Note: the presence of small amount of un-lysed cells would not affect the quality of the samples.

4. Transfer/pour the cell lysate to pre-chilled filter cartridge(s) in collection tube(s) and centrifuge in a microcentrifuge for 30 seconds at top speed (14,000-1,6000 X g).
5. Immediately place the collection tube on ice. Discard the filter cartridge according to your institution's waste disposal protocol. The cell lysate is now ready for downstream applications.

Table 1 Lysis Buffer Volume for Different Packed Cell Volumes*

Packed （ul）	Cell （ul）	Volume # X 10^6
3	20	0.3
5	50	0.5
10	100	1
20	200	2
40	500	3

*For NIH3T3 and 293T cells 10 µl packed cell volume is equivalent to about 10^6 cells

B. Adherent cells

1. Prior to protein extraction pre-chill the protein extraction filter cartridge (placed in collection tube) on ice.
2. Grow adherent cells to 90-100% confluence and wash the cells once in the tissue culture plates, dishes or flasks with cold PBS, aspirate the buffer completely.
3. Add appropriate amounts of cell lysis buffer (Table 2, this is a general reference volume, the actual amount of lysis buffer can be more or less), Scrape the lysed cells with a pipette tip or a transfer pipette and pipette up and down repeatedly to lyse the cells. Transfer the cell lysate to pre-chilled protein extraction filter cartridge(s) in collection tub(s). Centrifuge at top speed (14,000-16.000 X g) in a microcentrifuge for 30 seconds.
4. Immediately place the collection tube on ice. Discard the filter cartridge according to your institution's waste disposal protocol. The cell lysate is now ready for downstream applications.

Table 2 Amounts of lysis buffer required for different amount of adherent cells

Containers	Approximate Cell#	Lysis buffer(μl)
24-well plate	0.1-0.2 Million	50
6-well plate	0.6-0.8 Million	200
25 cm^2 flask	1.5-2 Million	500

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Following procedures are for 15-20 mg starting animal tissues. If smaller or larger amount of starting material is used adjust the amount of cell lysis buffer proportionately.

1. Prior to protein extraction pre-chill the protein extraction filter cartridge in collection tube on ice.
2. Place 15-20 mg fresh/frozen tissue in the filter. Grind the tissue with a plastic rod for 50-60 time with twisting force, add 200 µl denaturing cell lysis buffer to the filter and continue to grind for 30-60 times. Note: The plastic rod is reusable. For cleaning, rinse it thoroughly with distilled water and dry it with paper towel.
3. Cap the filter and incubate at room temperature for 1-2 min. Centrifuge at a microcentrifuge at top speed for 1-2 min. The supernatant of flow through contains denatured total protein extract.

Important Note: the presence of small amount of un-lysed tissue would not affect the quality of the sample.

柱式法 WB 专用
动物细胞和组织总蛋白提取试剂盒

描述：

柱式法动物细胞/组织总蛋白提取试剂盒，是新一代超快速蛋白质提取工具。越来越多的证据表明最常用的 RIPA 缓冲液可能导致蛋白质的随机丢失，产生很多难以解释的疑难数据。本试剂盒使用离心管柱提取技术结合优化的裂解缓冲液可以更快速，更有

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效地提取总蛋白，使 WB 结果更加准确。使用离心管柱技术提取蛋白，提取体系最低可以低至 20μl——有效解决小样本量的样本。

应用：

本试剂盒可用于动物细胞和组织的总蛋白样品制备，适用于 SDS-PAGE, WB 等下游应用。

试剂盒组分

1. 25ml 变性细胞裂解液
2. 50 个离心管柱
3. 50 个收集管
4. 2 根塑料研磨棒

**注：上述细胞裂解缓冲液不含任何还原剂和伯胺

运输储存：

常温

注意事项

1. **蛋白酶抑制剂不是必须加入，但是如果下游实验需要较长时间或者蛋白提取后保存较长时间，建议添加蛋白酶抑制剂。推荐使用 BCA 试剂盒用于蛋白浓度测定。研究蛋白磷酸化，磷酸酶抑制剂（例如 罗氏的磷酸酶抑制剂）应在使用前加入裂解缓冲液。**
2. **做 WB 上样前，仍需和 loading buffer 混匀煮制样品。**

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所需附加材料

1 X PBS
涡旋震荡仪
台式离心机
BCA 蛋白定量试剂盒

操作方法：

细胞样品总蛋白提取

A．非贴壁细胞

1.将离心管柱及接收管套管放在冰上预冷。

2.低速离心收集细胞，在 1.5ml 离心管中加入预冷的 PBS，旋窝震荡,500xg 离心 2-3 分钟清洗细胞。吸去上清，剩余与细胞体积相同体积的 PBS。涡旋震荡重悬细胞。

3.加入表格 1 中相应体积的细胞裂解液，涡旋震荡裂解细胞。**（细胞数量和裂解液须保证对应关系，以达到最佳提取效率）请注意：部分未完全裂解的细胞不会影响样品质量。**

表格 1，不同细胞体积应加入相应体积裂解液

细胞体积（ul）	裂解液（ul）	相当细胞量# X 10^6
3	20	0.3
5	50	0.5
10	100	1
20	200	2
40	500	3

* **NIH3T3 和 293T 细胞 10ul 体积相当于 1X10^6 个细胞**

4.将裂解的细胞转移到预冷的离心管柱套管中，14000-16000xg 离心 30 秒取出。

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5.立刻将收集管放置于冰上，弃去离心管柱，蛋白提取完成可应用于下游实验（**或及时转移至合适的样品管中-80℃保存**）。

B．贴壁细胞

1.将离心管柱及接收管套管放在冰上预冷。

2.将预冷的 PBS 直接加入培养板，培养皿或培养瓶中清洗贴壁细胞，吸去上清。

3.按照表 2 中将相应体积的细胞裂解液均匀的加入整个器皿表面，用移液器吹打几次，将裂解的细胞转移到预冷的离心管柱套管中，14000-16000xg 离心 30 秒取出。**（如提取浓度不佳，可减少裂解液使用量）**

4.立刻将收集管放置于冰上，弃去离心管柱，蛋白提取完成可应用于下游实验（**或及时转移至合适的样品管中-80℃保存**）。

表格 2，不同贴壁细胞量应加入相应体积裂解液

器皿	细胞数量	裂解液（ul）
24 孔板	0.1-0.2 Million	50
6 孔板	0.6-0.8 Million	200
25 cm2 培养瓶	1.5-2 Million	500

动物组织总蛋白提取

以下步骤是从 15-20mg 组织中提取。如果起始量较大或者较小，需调整相应裂解液的用量比例。

1.将离心管柱及接收管套管放在冰上预冷。

2.将 15-20mg 新鲜/冷冻组织放置于离心管柱上，用塑料棍向下扭转反复研磨 50-60 次，加入 200ul 细胞裂解液，继续研磨 30-60 次。**（组织用量不要过量，无需过度研磨，裂解液可分两次加入以得到最佳效果）**注意：塑料研磨棒可以重复使用，用蒸馏水彻底冲洗干净，用纸巾擦干。

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3.盖上盖子室温孵育 1-2 分钟，14000-16000xg 离心 1-2 分钟取出。接收管里的上清是抽提的总蛋白。蛋白提取完成可应用于下游实验（**或及时转移至合适的样品管中-80℃保存**）。

请注意：部分未完全裂解的组织不会影响样品质量。

常见问题

问题	解决方案
裂解物太粘稠，无法用 200-1000μL 吸头吹打	将细胞裂解物倒入离心管柱中或将吸头剪掉尖端
离心 30 秒后离心管中还存留细胞裂解液	减少起始细胞/组织的数量或增加细胞裂解液
低蛋白浓度	增加起始细胞/组织的数量或减少细胞裂解液量
高分子量范围（100-300KDa）蛋白条带弱	增加细胞裂解液确保细胞/组织裂解充分