Cell lysis buffer for IP (without inhibitors)

货号：RM00022

规格：100 mL

产品描述

本产品为 NP-40 裂解液，NP-40 裂解液是一种比较温和的细胞组织裂解液。裂解得到的蛋白样品可以用于常规的 Western、IP 和 Co-IP 等实验。裂解液中不含蛋白酶抑制剂，需根据要求选择添加蛋白酶抑制剂及磷酸酶抑制剂。当用于研究磷酸化作用使蛋白改良时，应加入磷酸酶抑制剂。如果出现目标蛋白降解，应加入蛋白酶抑制剂。用该裂解液裂解得到的样品，可用 BCA 或 Braford 法测定浓度。

产品成分

氯化钠（NaCl）：136.89mM；磷酸氢二钠（Na_2HPO_4）：1.01mM；

磷酸二氢钾（KH_2PO_4）：1.76mM；氯化钾（KCl）：2.68 mM

SDS：0.347 mM；NP-40：0.5%（V/V）

保存条件

-20℃保存，12 个月有效。

操作说明（仅供参考）

一、贴壁培养细胞

1、取 NP-40 裂解液室温溶解混匀，根据需要选择添加或不添加蛋白酶抑制剂。

2、去除贴壁细胞的培养液，用 PBS、NS 或无血清培养基清洗 1 次，低速离心，弃上清，留取沉淀。

3、按照 6 孔板每孔加入 100~200μL 裂解液的比例，加入 NP-40 裂解液，冰上裂解。移液器轻轻吹打，使裂解液和细胞充分接触。通常裂解液作用于细胞 1~5s 内，细胞会被裂解。

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4、1000~12000g，离心 3~5min（如果用冷冻离心机 4℃效果更佳），取上清。

5、进行后续的 SDS-PAGE、Western、免疫沉淀和免疫共沉淀等操作。

二、悬浮培养细胞

1、取 NP-40 裂解液室温溶解混匀，根据需要选择添加或不添加蛋白酶抑制剂。

2、低速离心悬浮细胞，弃上清，收集沉淀。

3、用手指轻弹细胞，使其松散。按照 6 孔板每孔加入 100~200μL 裂解液的比例，加入 NP-40 裂解液，冰上裂解。通常 6 孔板每孔加入 100~200μL 裂解液足够，但如果细胞密度非常高可以适当加大裂解液的用量 150~200μL，再用手指轻弹以充分裂解细胞。充分裂解后应无明显沉淀。

4、1000~12000g，离心 3~5min（如果用冷冻离心机 4℃效果更佳），取上清。

5、进行后续的 SDS-PAGE、Western、免疫沉淀和免疫共沉淀等操作。

三、组织样本

1、取 NP-40 裂解液室温溶解混匀，根据需要选择添加或不添加蛋白酶抑制剂。

2、把组织剪切成细小的碎片，越小越好。

3、取液氮或超低温冰箱中冷冻 30min 以上的组织，迅速用液氮研磨，研磨过程尽量控制在 1~2min 之内，以减少蛋白的降解。

4、按照每 20mg 组织加入 100~200μL 裂解液的比例，加入含有 PMSF 的裂解液。冰上裂解 30-60min。（步骤 3、4 也可采用以下过程：按照每 20mg 组织加入 100~200μL 裂解液的比例加入 NP-40 裂解液。用玻璃匀浆器或组织研磨器匀浆，直至充分裂解，过程尽量控制在 1~2min 之内，以减少蛋白的降解。）

5、1000~12000g，4℃离心 10~ 15min（如无低温离心机，室温下离心也可），取上清。

6、进行后续的 SDS-PAGE、Western、免疫沉淀和免疫共沉淀等操作。

注意事项

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1、去除贴壁细胞的培养液时，如果血清中的蛋白没有干扰，可以不用清洗。

2、如果裂解不充分可以适当增加裂解液的用量，如果需要高浓度的蛋白样品，可以适量减少裂解液的用量，尽量控制在冰上裂解。

3、如果细胞量较多，必需分装成 50~ 100 万细胞/离心管，然后再裂解。大团的细胞较难裂解充分，而少量的细胞由于裂解液溶液与细胞充分接触，相对比较容易裂解充分。

4、如果组织样品本身比较细小，可以适当剪切后直接加入裂解液溶解，再通过强烈 Vortex 使样品裂解充分。再离心取上清，用于后续实验。

5、细胞裂解的操作步骤，应置于冰上或 4℃进行。

6、本产品可作为免疫沉淀和免疫共沉淀的 Washing buffer 使用，每 200ug 磁珠加入 1mL 本产品进行洗脱即可。

Cell lysis buffer for IP (without inhibitors)

Catalog number: RM00022

Volume: 100 mL

Product Introduction

This Buffer is a mammalian whole cell lysis buffer based on a modified RIPA buffer formulation (the Ingredients are shown as follows). This Lysis Buffer is compatible with BCA Protein Assay or Bradford Protein Assay. The extracted protein samples with this lysis buffer can be used in Western Blot, IP and Co-IP, etc. This lysis Buffer does not contain protease or phosphatase inhibitors. If desired, add Protease and Phosphatase Inhibitor Cocktail immediately before use.

Reagents of Buffer

（Ingredients and final Concentration）

NaCl 136.89mM；Na_2HPO_4 1.01mM；KH_2PO_4 1.76mM；KCl 2.68 mM；SDS 0.347 mM

NP-40 0.5%（V/V）

Storage

Can be stored at - 20℃ within 12 months.

Product Usage Information

Thaw this lysis buffer at 24-30℃, mixing end-over-end.

Aliquot proper amount of buffer based on cell numbers and add Protease and Phosphatase Inhibitor Cocktail immediately before use.

For lysis of adherent cells, we recommend the following: (all reagents and lysates must be kept cold)

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1.Carefully wash cultured cells with pre-chilled PBS for 2 times.

2.Add in pre-chilled cell lysis buffer with Protease and Phosphatase Inhibitor Cocktail (1mL for 10_7cells, 100~200μL for one well of 6-well plate).

3.Scrap cells off to clean 1.5mL tubes with a clean, cold scraper. Put them on a low-speed rotating shaker for 15 min at 4°C.

4.Centrifuge at 12,000 g 4°C for 5min, transfer the supernatant to new tubes immediately. 5.The supernatants are extracted protein samples, which can be used in Western Blot, IP and Co-IP, etc. after measuring protein concentration.

For lysis of non-adherent cells, we recommend the following: (all reagents and lysates must be kept cold)

1.Collect cell pellets by centrifugation (1000g) and remove residual culture media.

2.Carefully wash cells by resuspending cells with pre-chilled PBS.

3.Collect cell pellets by centrifugation (1000g) and remove residual PBS.

4.Repeat step 2 and 3.

5.Add in pre-chilled cell lysis buffer pre-chilled cell lysis buffer with Protease and Phosphatase Inhibitor Cocktail (1mL for 10_7cells) and mix by pipetting.

6.Put them on a low-speed rotating shaker for 15 min at 4°C.

7.Centrifuge at 12,000 g 4°C for 5min, transfer the supernatant to new tubes immediately. 8.The supernatants are extracted protein samples, which can be used in Western Blot, IP and Co-IP, etc. after measuring protein concentration.

For lysis of tissue samples, we recommend the following: (all reagents and lysates must be kept cold)

1.Place tissue sample in a 60 mm or 100 mm dish and finely mince using a clean scalpel or razor blade. Keep dish on ice. It is important to keep the tissue cold to avoid protein degradation.

2.Collect the tissue sample pieces in 1.5mL tude and add pre-chilled cell lysis buffer with Protease and Phosphatase Inhibitor Cocktail (100~200μL for 20mg tissue).

3.Homogenize the tissue lysates by Dounce Homogenizer or other Homogenizers on ice as soon as possible.

4.Centrifuge at 12,000 g 4℃ for 5min, transfer the supernatant to new tubes immediately. The supernatants are extracted protein samples, which can be used in Western Blot, IP and Co-IP, etc. after measuring protein concentration.

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