BIO BASIC
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Product information

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FFPE Protein Extraction Kit

Catalog #: BSP058
Size: 50 Prep
Storage: 4°C*

*: Product will be shipped at ambient temperature. Upon receipt, store DTT powder and Extraction Buffer at 4°C. Pretreat Reagent 1, 2 and Precipitation Reagent are volatile. Tighten the lids and store at 4°C. Components will have a one year shelf life under these conditions.

Product Description:

In vitro cultures may not represent true differential protein expression patterns, as a result, in vivo, formalin-fixed, paraffin-embedded tissues represent a comprehensive source of protein expression profiles especially in clinical studies or studies of diseases such as cancer. These samples enable the course of disease — e.g., before and after therapy — to be examined . FFPE Protein Extraction Kit provides optimized conditions for extracting total protein from FFPE or formalin-fixed tissue sections. After rapid 5-minute de-paraffinizing by de-paraffinization reagent, the samples are incubated in an optimized extraction buffer at two different temperatures in a process that reverses formalin crosslinking and untangles the protein molecules. After a centrifugation step, the supernatant containing the released proteins is precipitated, recovered and can be used for Western Blot , SDS-PAGE and other applications. The kit can perform 50 mini-preps.

Contents:

Description	Size
Pretreat Reagent 1	100ml
Pretreat Reagent 2	5ml
Extraction Buffer	5ml
Precipitation Reagent	75ml
DTT	80mg

Note:

Before use, add DTT powder into Extraction buffer. Vortex until solution is completely dissolved. After usage, store Extraction Buffer at 4°C.

Procedure:

1. Transfer 2 sections, each with a thickness of 10-15 µm and an area of 100 mm^2 per preparation into a new 1.5ml centrifuge tube. Add 1ml Pretreat Reagent 1, vortex at high speed, and then keep at room temperature for 5 minutes. Invert the tube occasionally several times.
2. Add 100 µl Pretreat Reagent 2, vortex for several seconds. Centrifuge at 10000rpm for 2 minutes, discard two layer liquid.
3. Wash deparaffined tissue with ddH_2O twice. Discard supernatant and keep tissue pellet.
4. Pipet 100 µl Extraction Buffer into the tube containing the tissue. Close the lid and mix by vortexing.
5. Incubate the tube on a heating block at 100°C for 20 min. After, briefly spin the tube to bring down all contents. Break the tissue section with plastic pestle.
6. Incubate the tube at 60°C for 2 hours with agitation at 100 rpm.

7. Centrifuge the tube for 15 min at 12,000rpm at 4°C. Transfer the supernatant containing the extracted proteins to a new 1.5 ml collection tube .

Note:

The supernatant containing the extracted proteins can be stored for up to 1 week at 4°C. For long-term storage, aliquot the extracted proteins and store at –20°C. Avoid repeated freeze-thaw cycles. Carry out protein assay with Non-interfering protein concentration determination kit (SK3071).

8. Add 1000 µl Precipitation reagent, vortex and keep at 4°C for ten minutes.

9. Centrifuge the tube for 15 min at 12000rpm at room temperature, discard supernatant and keep pellet.

10. Add 500 µl Precipitation reagent, Repeat steps 8-9 once more. Invert the tube on filter paper and let liquid be drained off and residual liquid evaporate off.

11. Dissove the pellet with desirable volume of buffer (such as PBS, TBS, SDS-PAGE Loading Buffer, 2D Sample Extraction Buffer), and centrifuge at 10000rpm for 10 minutes at 4°C. Collect supernatant for quantization, SDS-PAGE.

Note:

Before dissolving pellet, add a little nuclease (such as micrococcal nucleas final concentration 1µ/100µl , benzonase final concentration 1µ/500µl) to digest DNA, RNA residues and reduce viscosity, further, desirable buffer containing urea, thiourea, chaps NDSB-201 or other detergent can help complete dissolution of protein pellet.

Additional Notes:

1. To optimize protein recovery, ensure the following criteria are met during fixation and embedding:
A. Fix tissue samples in 4-10% formalin as quickly as possible after surgical removal;
B. Use a fixation time of 12-24 hours (longer fixation times will result in poor protein extraction efficiency);
C. Thoroughly dehydrate samples prior to paraffin embedding.

2. Suitable starting materials are FFPE tissue samples cut directly from an FFPE sample block or unstained FFPE sections mounted on a microscope slide (e.g., sections from a series of FFPE tissue sections that could be used for histological or immunohistological analysis but have not been stained, for example with hematoxylin/eosin).

3. If using formalin-fixed tissue as starting materials, a 10 mg tissue piece can be used for one extraction and carry out step 3-10.

4. If using extracted total protein for ELISA, RIA experiments, standard antigen retrieval procedure can be carried out for good results. Western Blot is usually not necessary.

5. Fresh material or a sample that has been prepared using shorter fixation times (12–24 hours) delivers better results than older samples or those that have been prepared using longer fixing times (>24 hours).