ITSI BIOSCIENCES

TOTAL PROTEIN ISOLATION FROM FORMALIN-FIXED AND PARAFFIN-EMBEDDED SPECIMENS USING THE *ITSIPREP*™ ToPI-F2 KIT* - International

IMPORTANT: K-0017-10 is a validated kit developed specifically to make the isolation of total proteins from Formalin-Fixed Paraffin Embedded (FFPE) specimens possible, easy, reproducible and convenient. Approximately 5,000 µg/mL – 10,000 µg/mL of total protein can be extracted from 5 sections (~10 microns thick each) obtained from samples fixed with 10% buffered formalin and stored for up to 5 years. Note that depending on the desired protein yield, type of tissue, formalin fixation procedure and duration of storage, it may be necessary to adjust the number of sections used and procedure to compensate for the inherent difference in tissue types and age of FFPE specimens. The reagent provided in the kit is sufficient for isolation of more than 100,000 µg of total proteins under optimal conditions. We recommend the use of tubes and buffers supplied by ITSI or provided by vendors certified by ITSI because poor quality reagents may negatively impact downstream applications. Exercise extreme caution when working with proteins and protect your protein sample from breakdown and contamination by wearing gloves and placing tubes on ice. Work with clean equipment and in a clean/enclosed environment to prevent the introduction of common airborne contaminants such as keratin. Do not let samples stand at room temperature longer than it is necessary to prepare samples for downstream analysis.

Read the procedure completely and assemble all materials needed before starting.

MATERIALS PROVIDED IN THIS KIT:

ITEM	SIZE	CATALOG #	STORAGE
Solution 1	1 x 0.5mL	K-0017-10.1	Rm. T.
Solution 2	3 x 1.0mL	K-0017-10.2	4°C
Centrifuge Tubes	20 x 2.0mL	K-0017-10.3	Rm. T.
Procedure			

MATERIALS REQUIRED but Not supplied:

1. 100% Ethanol.
2. Xylene.
3. Thermostated Heating Device(s).
4. Chemical Safety Hood.

BEFORE STARTING:

- Before starting: Add 9.5mL of 100% Ethanol to **Solution 1** to make a total volume of 10mL of **Solution 1** to obtain 10ml of working buffer. Mix well.

PROCEDURE:

1. Transfer up to 5 FFPE tissue sections (8-10 microns tick each) to the screw cap centrifuge tube provided.
2. Add 1mL of **xylene (not provided)**.
3. Vortex for 10 – 15 seconds. Make sure that the sections are covered by the Xylene.
4. Let stand at room temperature for 5 minutes.
5. Centrifuge at 10,000 xg for 10 minutes.
6. Remove all supernatant with a pipette without disturbing the pellet. **Discard supernatant.**
7. Repeat steps 2 through 6 one more time.
8. Add 1mL of **Solution 1.**
9. Vortex briefly (~10 seconds).
10. Let stand at room temperature for 5 minutes.
11. Centrifuge at 10,000 xg for 10 minutes.
12. Remove all supernatant with a pipette without disturbing the pellet. Discard supernatant.
13. Re-centrifuge for 30 seconds and carefully remove any remaining supernatant.
14. Dry pellet for 3 minutes under a hood.
15. Add 100 µL of **Solution 2**.
16. Incubate for 20 minutes at 100°C.
17. Incubate further for 2 hours at 60°C.
18. Vortex briefly (about 4 times) during the 2-hour incubation.
19. Cool to room temperature.
20. Centrifuge at 10,000 xg for 15 minutes.
21. Transfer supernatant to a fresh tube. **This fraction contains the isolated proteins.**

Note: Solution 2 contains SDS. Remove SDS if it will interfere with the desired downstream step. Precipitate/concentrated proteins e.g. with ToPREP (Cat #: K-0016) if a more concentrated sample is desired.

STORAGE:

ITSI recommends that protein samples be stored at –80°C in appropriate tubes until analyzed.

*CONDITIONS FOR USE OF THIS PROCEDURE/SOLUTIONS:

This VBP is the intellectual property of ITSI Biosciences. Only complete set of reagents provided by ITSI Biosciences should be used when possible because their compatibility with the downstream application has been validated. Considering that many factors can cause experiments to fail, ITSI Biosciences cannot guarantee that the use of this VBP and buffers will lead to a successful experiment. In no event shall ITSI Biosciences be held liable for loss of samples, failure of experiments or any other damage or injury associated with the use of this procedure or associated materials and reagents.

General Safety Information:

Consider all chemicals as potentially hazardous. Only trained laboratory personnel familiar with good laboratory practice should handle this product. Protective clothing should be worn. Use caution to avoid contact with skin and eyes. If contact should occur, wash immediately with water and follow established guidelines/procedures in your laboratory. **WARNING: Intended for research use only, not for use in human, therapeutic or diagnostic applications. The end user is responsible for all local, state and federal regulations associated with the use and disposal of laboratory reagents.**

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