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| **Document Number:** | **SASoM/METHOD/069.v4** |
| **Title:** | **Protein Extraction from FFPE Sample (Paraffin) Blocks for RPPA analysis.** |
| **Version:** | **v4** |
| **Author:** | **Peter Mullen** |

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|---|---|
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| **SOP History** | | |
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| Number | Date | Reason for Change |
| v1 | 19/08/2014 | Original |
| v2 | 08/08/2016 | Update |
| V3 | 08/08/2018 | Update |
| V4 | 11/11/2020 | Update |
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**1.0 Purpose –**
This SOP describes the current procedure for extracting protein extractions from FFPE paraffin blocks in Laboratory 248 at the St Andrews School of Medicine (SASoM).

**2.0 Scope –**
This SOP applies to the staff in the SASoM involved in preparing protein extractions from FFPE Sample Blocks.

**3.0 Responsibilities –**
All staff involved in cell culture are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.

**4.0 Procedure –**
This protocol uses the Qiagen 'Qproteome FFPE Tissue Kit' as detailed below:

| Qproteome FFPE Tissue Kit | (20) |
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| Catalog no. | 37623 |
| Number of preps | 20 |
| Extraction Buffer EXB Plus | 2 x 1 ml |
| Collection Tubes (1.5 ml) | 50 |
| Collection Tube Sealing Clips | 20 |
| Handbook | 1 |



*Equipment and Reagents: Microcentrifuge (with rotor for 1.5 ml tubes); Heat Block, Thermomixer, Vortexer, Xylene, 100%, 96%, and 70% (v/v) ethanol**

** Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.*

*All centrifugation steps are carried out at room temperature (20–25°C) using a bench-top microcentrifuge!*

*NB. Xylene washes should be performed in a fume hood!*

**Procedure – (A) Deparaffinization of FFPE Tissue Sections**

1. Cut 4 x serial 15-20 µm thick sections from the same block. Additional sections (4 µm) may also be taken for histological confirmation
2. Immediately place the sections in a 1.5 ml microcentrifuge collection tubes (supplied in kit). Samples can be stored at -20°C prior to processing.
3. Pipet 1 ml xylene into the tube ☐. Vortex vigorously for 10 s and incubate for 10 min.
4. Centrifuge the tube in a microcentrifuge at full speed for 3 min. Carefully remove and discard the xylene supernatant for safe disposal.

5. Repeat steps 3 and 4 twice ☐ ☐.
6. Pipet 1 ml of 100% ethanol ☐ into the tube containing the pellet and mix by vortexing. Incubate for 10 min. Centrifuge the tube at full speed for 3 min.
7. Carefully remove and discard the supernatant.
8. Do not disturb the pellet.
9. Repeat steps 6 and 7 ☐.
10. Pipet 1 ml of 96% ethanol ☐ into the tube containing the pellet and mix by vortexing. Incubate for 10 min. Centrifuge the tube at full speed for 3 min.
11. Carefully remove and discard the supernatant.
12. Do not disturb the pellet.
13. Repeat steps 10 and 11 ☐.
14. Pipet 1 ml of 70% ethanol ☐ into the tube containing the pellet and mix by vortexing. Incubate for 10 min. Centrifuge the tube at full speed for 3 min.
15. Carefully remove and discard the supernatant.
16. Do not disturb the pellet.
17. Repeat steps 14 and 15 ☐. Note: *If necessary, repeat the centrifugation step to enable removal of any residual ethanol. Do not disturb or remove any of the pellet.*
18. Proceed immediately to the next step of the protocol – (B) Protein Extraction.



**Procedure – (B) Protein Extraction**

19. Make up 'Complete Extraction Buffer EXB Plus' by adding 6.6 µl of β-mercaptoethanol to 103.4µl of Qproteome Extraction Buffer EXB Plus (100µl per sample with 10% extra for pipetting). Other volumes can be made up as follows:
    a. 6.6 µl of β-mercaptoethanol to 103.4µl of Qproteome Extraction Buffer (n=1)
    b. 13.2 µl of β-mercaptoethanol to 206.8µl of Qproteome Extraction Buffer (n=2)

c. 19.8 µl of β-mercaptoethanol to 310.2µl of Qproteome Extraction Buffer (n=3)

d. 26.4 µl of β-mercaptoethanol to 413.6µl of Qproteome Extraction Buffer (n=4)

e. 33 µl of β-mercaptoethanol to 517µl of Qproteome Extraction Buffer (n=5)

f. 39.6 µl of β-mercaptoethanol to 620.4µl of Qproteome Extraction Buffer (n=6)

20. Pipet 100 µl Extraction Buffer EXB Plus supplemented with β-mercaptoethanol into the tube containing the excised tissue or pellet and mix by vortexing. Seal the Collection Tube with a Collection Tube Sealing Clip (supplied).

21. Incubate on ice for 5 min, and mix by vortexing. Note: Be sure that Collection Tubes are properly sealed with a Collection Tube Sealing Clip before performing the next step.

22. Incubate the tube on a Heating Block at 100°C for 20 min.

23. Using a Thermomixer, incubate the tube at 80°C for 2 h with agitation at 750 rpm.

24. After incubation, place the tube at on ice for 1 min and remove the Collection Tube Sealing Clip. Note: Be sure that Collection Tube Sealing Clip has been removed before starting the centrifugation step.

25. Centrifuge the tube for 15 min at 14,000 x g at 4°C. Transfer the supernatant containing the extracted proteins to a new 1.5 ml Collection Tube and label appropriately.

26. Perform a '**Biorad RC-DC protein assay**' as per the prescribed SOP (SaSoM/METHOD/070-Protein determination in 1.5mL microcentrifuge tubes using the Biorad RC-DC protein assay) – it is crucial that proteins are measured using this assay as both the BCA and Bradford protein assays are not compatible with the detergents and β-mercaptoethanol which are present in the Qiagen lysis buffer.

27. Store samples at -80°C prior to use.



**5.0 Personal protection -**

A Howie laboratory coat and lab gloves must be worn at all times.

**6.0 Spillages -**

Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard.

Spillages should be mopped up with paper towel, disinfected with 70% ethanol and finally washed with water.

Any spillages of Xylene must be mopped up with tissues which are evaporated dry inside the fume cabinet before being placed in a sealed bag for disposal into the red chemical waste bins.

**7.0 Training -**
All staff should be properly trained before beginning this work.

**8.0 Related documents –**

Risk assessments COSHH/011
RA/GEN/019

8.2 SOP SASoM/EQUIP/029
Use and Maintenance of the Biohit BP800 Microplate Reader



**9.0 Approval and sign off –**

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