STORE

SOP Title:

Preparing FFPE pellets from cell lines

The STORE processing methods were shown to be fit-for purpose for DNA, RNA and protein extraction from FFPE material. The STORE characterisation methods were shown to be fit for-purpose for Quality Control of the extracted DNA, RNA and proteins.

HUMAN CANCER STUDIES GROUP

SOP reference **FP01**

Standard Operating Procedure for

Preparing FFPE pellets from cell lines

Version number ……1……

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Author:

Name ……Dr B Mathieson……..

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Purpose:

Preparation of formalin fixed paraffin embedded pellets from cell lines for controls for FFPE extraction protocols.

Materials

1. One TripleFlask (Nunc) or three 175 cm^3 culture flasks at approximately 80% confluence (a triple flask contains 500cm^3 of cell culture surface area).
2. Human plasma (Sigma P9523-1ml)
3. Bovine thrombin (Sigma T9468-1 KU or Sigma T6200-1 KU)
4. 50ml centrifuge tube
5. PBS
6. 15 ml centrifuge tube
7. 2ml Safelock Eppendorf tube
8. 21G (green) needle.
9. Biopsy capsule

Protocol

1. Make up the plasma by adding 1ml ddH_2O into the vial and solubilising the lypholyzed content. Aliquot into 200µl aliquots and store those not immediately required at -20ºC for future use.
2. Make up the thrombin by adding 2ml ddH_2O into the vial and solubilising the lypholyzed content. Aliquot into 350µl aliquots and store those not immediately required at -20ºC for future use.
3. Recover cells from the culture flask(s) using the appropriate disassociation medium.
4. Gently pour the cell suspension into a 50ml centrifuge tube, top up with culture medium (containing FCS if trypsin has been used in the disassociation buffer). Centrifuge at 200*g* (approximately 850rpm) for 5 min.
5. Remove the supernatant and gently re-suspend the cell pellet in 1.5ml of warmed PBS.
6. Transfer the cell suspension to a 15ml centrifuge tube, fill with warmed PBS and invert the tube five times to wash the FCS from the cells. Centrifuge at 200*g* (approximately 850rpm) for 5 min.
7. Remove the supernatant then gently re-suspend the cell pellet in 1.5ml of warmed PBS.
8. Transfer the cell suspension to a 2ml Safelock Eppendorf tube and centrifuge at 400*g* (approximately 2000rpm) for 20 sec.
9. Remove the supernatant, add 200µl of plasma and gently use the pipette to create a single cell suspension.
10. Add 350µl of thrombin and use the pipette of mix the thrombin, plasma plus cells together.
11. Allow the pellet to clot (5 - 10 min).
12. Insert a needle between the clot and the side of the Safelock tube and traverse the circumference of the eppendorf to dislodge the pellet.
13. Place a biopsy capsule onto of a sheet of absorbent paper, invert the Safelock tube and gently tap it in the centre of the biopsy capsule. The pellet will fall into the capsule but remain intact.

14. Close the biopsy capsule, soak it in 10% buffered formalin solution for 12-24 hours then process and embed it using a standard protocol (Histopathology Department).