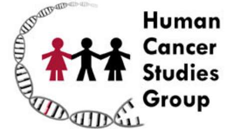
SOP Title:

Preparation of FFPE sections prior to processing to nucleic acid

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



Standard Operating Procedure for

Preparation of FFPE sections prior to processing to nucleic acid

Date created ………2/9/2010

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Summary

This protocol explains the procedure for the preparation of whole tissue sections prior to extraction of nucleic acid. There are 3 versions – A for sections placed in eppendorfs, B for sections mounted on slides and C for sections mounted on glass slides that require macrodissection to remove unwanted tissue.

Safety Considerations

Protective clothing and gloves should be worn throughout

Xylene is hazardous by inhalation – use in a fume hood

Materials

- xylene
- methanol
- ethanol (absolute, 96%, 70%)
- Nuclease-free water (if not in kit)
- Scalpel
- 1.5ml eppendorf

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Method A for sections in an eppendorf

1. Place on ml xylene into eppendorf with sections at room temperature for 5 minutes
2. Centrifuge eppendorf at 13,000 rpm for 5 minutes
3. Carefully remove supernatant
4. Repeat steps 1,2 and 3 twice
5. Add 1ml methanol to eppendorf containing sections at leave at room temperature for 5 minutes.
6. Centrifuge eppendorf at 13,000 rpm for 5 minutes
7. Carefully remove supernatant
8. Repeat steps 5-7 once more
9. Add 1ml absolute ethanol to eppendorf containing sections at leave at room temperature for 1 minute.
10. Centrifuge eppendorf at 13,000 rpm for 5 minutes
11. Carefully remove supernatant
12. Add 1ml 96% ethanol to eppendorf containing sections at leave at room temperature for 1 minute.
13. Centrifuge eppendorf at 13,000 rpm for 5 minutes
14. Carefully remove supernatant
15. Add 1ml 70% ethanol to eppendorf containing sections at leave at room temperature for 1 minute.
16. Centrifuge eppendorf at 13,000 rpm for 5 minutes
17. Carefully remove supernatant
18. Leave cap open of eppendorf and leave to dry at room temperature for 10 minutes
19. Proceed to extract desired nucleic acid using appropriate protocol

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Method B for sections mounted on glass slides

- Use sections mounted on uncoated glass slides where possible – slides should be dried overnight at room temperature. This protocol works with any section thickness up to 20 μm

1. Place the slide in xylene at room temperature for 5 minutes
2. Remove slide and place slide in fresh xylene for a further 5 minutes
3. Repeat step 2 again
4. Remove slide from xylene and place in methanol for 5 minutes.
5. Remove slide and place slide in fresh methanol for a further 5 minutes
6. Remove slide and place in absolute ethanol for 30 seconds
7. Remove slide and place in 95% ethanol for 30 seconds
8. Remove slide and place in 70% ethanol for 30 seconds
9. Remove the slide from the ethanol, and allow to dry briefly.
10. Wet the tissue on the slide with a small amount of nuclease free water (~10-20ul). Carefully remove most of the water, without disturbing the tissue.
11. Use the scalpel to scrape the wet tissue into a ball, and deposit in a 1.5ml eppendorf.
12. Proceed to extract desired nucleic acid using appropriate protocol

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Method C for sections mounted on glass slides that require macro-dissection

- Use sections mounted on uncoated glass slides where possible – slides should be dried overnight at room temperature.
- A H&E section of each sample is required, with the area of sample (ie tumour) to be macro-dissected marked

1. Using the H&E reference slide, mark the underside of the sample slide, to highlight the area to be macro-dissected.
2. Scrape off the unwanted area using a scalpel (clean the scalpel in 70% Ethanol between cases), leaving on the region for extraction on the slide.
3. Place the slide in xylene at room temperature for 5 minutes
4. Remove slide and place slide in fresh xylene for a further 5 minutes
5. Repeat step 2 again
6. Remove slide from xylene and place in methanol for 5 minutes.
7. Remove slide and place slide in fresh methanol for a further 5 minutes
8. Remove slide and place in absolute ethanol for 30 seconds
9. Remove slide and place in 95% ethanol for 30 seconds
10. Remove slide and place in 70% ethanol for 30 seconds
11. Remove the slide from the ethanol, and allow to dry briefly.
12. Wet the tissue on the slide with a small amount of nuclease free water (~10-20ul). Carefully remove most of the water, without disturbing the tissue.
13. Use the scalpel to scrape the wet tissue into a ball, and deposit in a 1.5ml eppendorf.
14. Proceed to extract desired nucleic acid using appropriate protocol

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