Isolation of Interphase Nuclei from Paraffin Section for FISH, Hedley Technique

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National Institutes of Health

Reagents

Ethanol, absolute
Protease Type XXIV, Bacterial
Sigma, Cat. P-8038
Phosphate Buffered Saline (PBS), 1X
Xylene
Water, sterile
Serum Filter Columns
Fisher Scientific,Cat. 1138755
DAPI
Sigma, Cat. D-9542
Sulpharhodamine SR101
Sigma, Cat. S-7635

Preparation

0.1% Protease
Dissolve 100 mg of protease in 1X PBS to a final volume of 100 ml

DAPI-Sulpharhodamine Solution
1 mM DAPI: dissolve 10 mg in 27 ml sterile water
5 mM Sulpharhodamine: dissolve 50 mg in 16.5 ml distilled water

<u>Combine for final solution:</u>

1X PBS, pH 7.4	92 ml
DAPI solution	2 ml
Sulpharhodamine (SR101) solution	2 ml

Procedure

One 50 micron section or two 30 micron sections from formalin fixed, paraffin embedded, breast tumors are suggested for use.

1. Outline desired area on slide using syringe needle and then scrape away excess wax/material with razor.

2. Place slides into coplin jars filled with xylene for 3 x 10 min.

3. Remove from xylene and then put through ethanol series 100%, 90%, 70%, and 50% for 5 min each.

4. Once done with washes carefully remove section from slide using syringe needle and place in eppendorf tube filled with 1 ml 50% EtOH.

5. Centrifuge for 15 min at 1400 rpm; remove EtOH.

6. Then allow to sit in 1 ml sterile water for 20 min at room temperature.

7. Centrifuge again for 15 min at 1400 rpm.

8. Remove water and add 500 µl of 0.1% Protease in 1X PBS and place in 45˚C shaking waterbath for 45-60 min.

9. After this time check nuclei by placing one drop of solution on slide and add one drop DAPI-Sulpharhodamine. Check for optimal disintegration of tissue sample in fluorescent microscope looking for:
   - Quantity: around 30 nuclei per 25X objective field view
   - Presence of cytoplasm: optimal with little to no cytoplasm and intact nuclei
   - Fluorescence intensity of DAPI-Sulpharhodamine: stronger nuclear intensity is better, indicating less protein

10. If the above three criteria are not optimal continue incubation in waterbath and check again after 30 min.

11. To stop reaction, add 500 µl 1X PBS to samples.

12. Filter each sample using a serum filter column.

13. Centrifuge 10,000 rpm for 5 min.

14. Gently remove supernatant leaving 100 µl, resuspend nuclei using vortex and add varying amounts of 1X PBS according to desired cell concentration.

15. Cytospin 80 µl nuclei suspension in Shanndon Cytospin. Spin for 3 min at 1600 rpm. Check concentration of nuclei with a phase contrast microscope and adjust concentration as desired.

16. Dehydrate slides in 70% and 90% EtOH for 5 min each, then 100% EtOH for 10 min.

17. Allow slides to air dry and then store at 4˚C.