Deparaffination and Antigen Retrieval of FFPE Tissues

Equipment and Materials:

Reagents:

Water: (H_2O), Milli-Q System Water; Ethyl Alcohol, 200 proof, Absolute Anhydrous ACS/USP Grade, Pharmco-AAPR 111000200; Xylenes, Reagent ACS, Acros 42268-0040;Trizma Base, minimum 99.9% titration, Sigma T1503

Equipment:

Digital Decloaking Chamber: Biocare Medical DC2002; Wheaton Glass Staining Dish for 5 slides, Fisher Scientific 08-813E; Plastic Coplin Staining Jar, Fisher Scientific 19-4

Procedure:

Reagent Preparation:

Stock solutions of Ethanol:

95% Ethanol: 475ml Ethanol, 25 ml Milli-Q H_20

70% Ethanol: 350ml Ethanol, 150 ml Milli-Q H_20

10mM Tris Buffer; pH 9.0

1.21 g Tris Base

1000 ml Milli-Q H_20

Completely dissolve Tris. The pH should be around 9.0, if not, adjust with NaOH or HCl.

Deparaffination Procedure:

Each step should be in a different coplin jar and slides should be completely submerged. EtOH and Mill-Q H_20 should be disposed of into chemical waste disposal bottles.

1. 3 min in Xylenes
2. 3 min in Xylenes
3. 1 min in 100% EtOH
4. 1 min in 100% EtOH
5. 1 min in 95% EtOH
6. 1 min in 70% EtOH
7. 3 min in Mill-Q H_20

8. 3 min in Mill-Q H_20

9. Slides can sit up-right after last step, placed directly into Tris Buffer for antigen retrieval (see 5.3.4 below), or stained (starting with the Hematoxylin step in the H&E protocol, MSRC-R-004, 5.2.4).

Antigen Retrieval Procedure:

1. Add 500 ml deionized water to the decloaking chamber.

2. Turn on the decloaking chamber.

3. Check settings on the decloaker display by pressing the "Display" button. The settings should be in this order:
    a. 95°C - temperature for chamber to reach
    b. 20 minutes - amount of time the chamber will heat samples at 95°C
    c. 90°C - temperature at which point the lid can be removed
    d. 10 seconds, when the chamber has steadily held the 90°C

4. Fill the plastic vertical staining dish with 10mM Tris. Place slides in this dish, making sure that the slides are completely submerged.

5. Place the staining dish into decloaker, slightly on the edge of the grate, so that the dish is not directly on the source of heat.

6. Place the lid on top of the staining dish, but do not tighten.

7. Make sure the gasket is in place. The gasket ensures an airtight chamber.

8. Place the decloaker lid on top of the decloaker and close. The dot on the handle must match up with the "closed" wording on the lid.

9. Press the start button to begin the programmed run.

10. It will take approximately 5 minutes for the chamber to reach 95° and then 20 minutes to maintain that temperature for a total wait time of 25 minutes for this part of the program. The pressure should remain below 5 psi.

11. Once the first part of the program has completed, the decloaker will repeatedly beep. Press start to continue to the next part of the program.

12. The chamber will now drop in temperature and pressure, 90° and 0 psi. Once it reaches these two points, the chamber will repeatedly beep again. Press the start button again. The decloaking chamber can now be turned off.

13. Toggle the petcock to make sure all pressure has been released. Now the lid can be taken off. Open away from you to avoid steam contact burns.

14. Using the silicone glove, carefully remove the staining dish and place it on the counter. Allow the dish to cool on the bench top for 10 minutes in the buffer.

15. Exchange the Tris Buffer with Milli-Q H_2O five times, pouring out half of the liquid each time, with the final rinse being entirely water.

16. Stand slides vertically on a paper towel to dry completely. Place slides in desiccator until needed.

Expected Outcome:

After deparaffination procedure, there should no longer be paraffin around the tissue. If the xylenes dish is diluted, some paraffin residue can still be seen on the slides. Please pour out diluted xylenes into the chemical waste disposal bottle and refill with fresh xylenes. Successful antigen retrieval will allow you to see peptide spectra once digested with trypsin.

References:

1. **BIO**CARE Medical. *Digital Decloaking Chamber-Operation Handbook.*
2. Protocol for Tissue Staining with Hemotoxylin and Eosin (H&E)