

Detection of CK34BetaE12 in Frozen Human Tissue Using the Ventana

Staining Procedure

-Positive Control Tissue: Prostate

-Stain Localization: Cytoplasmic

Cut frozen sections and immediately fix in Rapid Fix (Shandon-Lipshaw) for 7 seconds.

Pull two slides from the box in the ?80 labeled 'Cytokeratin controls'
Lie slides flat and allow to warm up to room temperature for 30 minutes.
Place slides in Rapid Fix for 7 seconds
Place slides in buffer

Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

Place slides in 0.3 % H2O2 for 30 minutes

Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

1. Perform Heat Induced Epitope Retrieval using decloaker.

Add 500 ml D/W to the pan of the decloaker.

Place a full rack of slides in citrate buffer and put in the decloaker.

Note: When using a Tissue Tek container, add 250 mls of 1X citrate buffer.

Decloak for 5 minutes. pressure _____

Depressurize for 10 minutes.

Remove pan top and cool for 10 min. temp _____

Rinse in D/W, 2x for 3 min each

2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

3. Place slides on Ventana

For CK,34betaE12 run Protocol #61

For Negative control slides run Protocol #49

Amplification Master Lot # _____

A/B Block Master Lot # _____

DAB Detection Kit Master Lot # _____

Ventana CK34BetaE12 Master Lot# _____

Ventana Negative Control Mab Master Lot# _____

Titration run ? 32 minute incubation

4. Upon completion of run, remove slides from machine and rinse in warm detergent water to remove Ventana coverslip from slides.

5. Rinse in tap water 3 minutes.

6. Counterstain with Modified Harris Hematoxylin for 45 seconds.
7. Rinse in tap water until water is clear.
8. Place slides in 1X Automation buffer for 1 minute with gentle agitation to blue slides.
9. Dehydrate through the following solutions

95% Ethanol	1 change	3 minutes
100% Ethanol	3 changes	3 minutes
Xylene	2 changes	5 minutes

Coverslip.

last updated 01/04/2004