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Antigen Retrieval Protocol – EDTA Buffer

For use with UltraPlex and UltraPolymer reagents

Protocol

1) Dewax slides as follows:

Xylene	5 min
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100% Reagent Alcohol	2 min
100% Reagent Alcohol	2 min
95% Reagent Alcohol	2 min

2) Wash slides with tap water 2 changes, 2 min each.
3) Wash slides with distilled water, 2 min. Perform antigen retrieval by placing slides in a staining container and steaming in a pressure cooker on high pressure (approximately 120°C) with 200ml of 1mM EDTA buffer, 0.05% Tween 20, pH 8 for 15 min.
4) Let slides cool in pressure cooker for 10 min before releasing pressure.
5) Release pressure and move slides to hot distilled water for 2 min.
6) Flush with running tap water for 5 min.
7) Proceed to staining protocol

Notes

1. EDTA antigen retrieval buffer (1mM EDTA, 0.05% Tween 20, pH 8.0) may be made from solid EDTA disodium salt dihydrate and stored as a concentrated 10X solution (10mM EDTA, 0.5% Tween 20) in dH_2O, pH 8.0.
Dissolve 3.7g of disodium EDTA dihydrate in 950ml distilled water then bring pH to 8.0 using 1N NaOH. Add 5ml Tween 20 and mix well before bringing final volume to 1000ml with distilled water. This solution can be stored for 3 months at room temperature or longer if stored at 4°C.

2. From the concentrated 10X solution, 1X working solution can then be prepared as needed by further dilution of the 10X stock in dH_2O. Buffer pH = 8.0 should be confirmed prior to each use, as pH levels may change during storage.

Disclaimer

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