



TintoDeparaffinator EDTA 20X





Intended Use

For Research Use Only.

Summary and Explanation

The TintoDeparaffinator EDTA solution is intended for paraffin removal from paraffin-embedded tissues, hydrating and heat permeabilizing tissues to achieve epitope or nucleic acid retrieval. The TintoDeparaffinator EDTA is an innovative reagent formulated to reduce exposure to toxic solvents and reduces the number of steps in deparaffinization, hydration and epitope or nucleic acid retrieval, thus making it a safe and efficient alternative to traditional deparaffinization involving the use xylene and alcohol. Since they do not interfere with the detection of proteins by Immunohistochemistry,

immunocytochemistry, or of nucleic acids by CISH and FISH, results are the same as when using xylene and alcohol.

The deparaffinization procedure with TintoDeparaffinator EDTA solution uses a heat-assisted deparaffinization method while achieving tissue hydration and retrieval of proteins and nucleic acids. Deparaffinization at moderately elevated temperatures has been shown to adequately dissolve paraffin at significantly lower solvent concentrations compared to methods of deparaffinization at ambient temperatures using xylenes and/or xylene substitutes.

By utilizing lower non-toxic solvent concentrations, the process of tissue rehydration following deparaffinization and retrieval can be achieved with a single final rinse step to remove micro-paraffin leftovers, followed by direct transfer of slides to a buffer bath. These reagents are specifically designed to work with the Bio SB TintoRetriever Pressure Cooker (BSB 7008) or TintoRetriever PT Module (BSB 7030 and BSB 7033), but may be used with any heating device that provides the required temperature.

These reagents are designed to deparaffinize, rehydrate and retrieve one microscope slide containing a standard size (from 0.5cm x 1cm up to 2.5cm x 2.5cm diameter) paraffin-embedded tissue section per 3 ml of TintoDeparaffiniator EDTA 1X. A typical staining dish containing 200-250 ml will deparaffinize approximately 72 standard tissue sections. If larger tissue sections are used, the number of processed slides may be less. If residual paraffin is observed microscopically on the slides following deparaffinization, the deparaffinization reagents should be replaced.

The TintoDeparaffinator Hot Rinse (BSB 0179 and BSB 0180) may be used as the final rinse step to remove residual micro-paraffin, but is optional. ChromoProtector (BSB 0152 and BSB 0153) is highly recommended to be used after the intended staining procedure in order to remove any significant micro-paraffin that may be leftover before mounting slide with a coverslip. For optimal results, it is recommended to use both TintoDeparaffinator Hot Rinse and ChromoProtector to remove all micro-paraffin.

Presentation

TintoDeparaffinator EDTA 20X contains glycols and glycol ethers. It is provided in liquid form.

Catalog No.	Concentration	Volume
BSB-0177-RUO	TintoDeparaffinator EDTA 20X	100 mL
BSB-0178-RUO	TintoDeparaffinator EDTA 20X	1L

Storage Store at 20-25°C

Stability

This product is stable up to the expiration date on the product label.

Do not use the after expiration date listed on package label. Temperature fluctuations should be avoided. Store appropriately when not in use. Adhere to all local laws when disposing of this product.

Precautions

- 1. For professional users only. Results should be interpreted by a qualified medical professional.
- 2. This product contains <0.1% sodium azide (NaN₃) as a preservative. Ensure proper handling procedures are used with this reagent.
- 3. Always wear personal protective equipment such as laboratory coat, goggles and gloves when handling reagents.
- 4. Dispose of unused solution with copious amount of water.
- 5. Do not ingest reagent. If reagent is ingested, seek medical advice immediately.
- 6. Avoid contact with eyes. If contact occurs, flush with large quantities of water
- 7. Follow safety precautions of the heating device used for epitope retrieval (TintoRetriever Pressure Cooker or similar).
- 8. For additional safety information refer to Safety Data Sheet for this product.
- 9. For complete recommendations for handling biological specimens, please refer to the CDC document, "Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories" (see References in this document).

Preparation of Working Solution

TintoDeparaffinator EDTA 20X must be diluted prior to use with distilled water to achieve a 1X working solution.

Dilute the solution 1:20, for example, to achieve 1 liter of 1X working solution would be to add 50 mL of TintoDeparaffinator EDTA 20X to 950 mL of distilled water.

Recommended Protocol

- $1.\,\mathrm{Dilute}$ the TintoDeparaffinator EDTA 20X solution 1:20 with distilled water.
- 2. Add the diluted TintoDeparaffinator EDTA 1X solution to a staining dish (200 mL, or enough to cover tissues).
- 3. Place the slides with mounted tissues or cells in a slide holder and then immerse them into the TintoDeparaffinator EDTA.
- 4. Optional: Add the TintoDeparaffinator Hot Rinse 1X to a second staining dish (200 mL, or enough to cover tissues).
- 5. Position both the staining dishes containing the slides with mounted tissues or cells in the TintoDeparaffinator EDTA 1X, and the TintoDeparaffinator Hot Rinse 1X into the heating apparatus.
- 6. Heat slides at a high pressure with a temperature of 114°C to 121°C for 10-15 mins. Other heat retrieval options are low pressure at 106°C to 110°C, or the PT module/water bath at 95°C to 98°C for 30-45 mins.
- 7. After allotted time has passed, remove both staining dishes from the heating apparatus. Leave to cool for 15- 20 mins at room temperature.
- 8. Remove slide rack with slides from TintoDeparaffinator EDTA 1X solution, and tap on a paper towel to remove as much of the solution as possible. Do not let slides dry out.
- 9. Transfer into TintoDeparaffinator Hot Rinse 1X solution for 5 minutes. 10. Remove from TintoDeparaffinator Hot Rinse solution, and tap on a paper towel to remove as much of the solution as possible.
- 11. Transfer to a room temperature buffer bath for a few minutes, and then proceed with IHC, ICC, CISH or FISH staining protocol.
- 12. After counterstaining, incubate slides in Chromoprotector at 60°C for 10 mins, and air dry completely before cover-slipping slides.

Abbreviated Immunohistochemical Protocol

Step	ImmunoDetector AP/HRP	PolyDetector AP/HRP	PolyDetector Plus HRP
Peroxidase/AP Blocker	5 min.	5 min.	5 min
Primary Antibody	30-60 min.	30-60 min.	30-60 min.
1st Step Detection	10 min.	30-45 min.	15 min.
2nd Step Detection	10 min.	Not Applicable	15 min.
Substrate- Chromogen	5-10 min.	5-10 min.	5-10 min.
Counterstain / Coverslip	Varies	Varies	Varies

Product Limitations

Due to inherent variability present in immunohistochemical procedures (including fixation time of tissues, dilution factor of antibody, retrieval method utilized and incubation time), optimal performance should be established through the use of positive and negative controls. Results should be interpreted by a qualified medical professional.

References

1. U.S. Department of Health and Human Services: Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. Supplement / Vol. 61, January 6, 2012.



