

Product Information

AntiFix™ Universal Antigen Retrieval Buffer, 10X

Catalog Number: 22030-50mL, 22030-500mL

Unit Size: 50 mL, 500 mL

Storage and Handling

Store at room temperature. Product is stable for at least 12 months from date of receipt when stored as recommended. The buffer is non-toxic and non-flammable.

Product Description

AntiFix™ Universal Antigen Retrieval Buffer is designed for heat-induced epitope retrieval (HIER) of formalin-fixed, paraffin embedded (FFPE) tissues. The buffer uses a proprietary reagent that catalyzes the reversal of formaldehyde cross-links in FFPE tissue to restore antigen reactivity.

Fixation of tissue with formalin (formaldehyde) preserves tissue morphology, but the cross-linking of proteins by formaldehyde can modify antibody binding sites or epitopes so they are no longer detectable by immunohistochemistry. Traditionally, epitope reactivity is recovered by using protease treatment, or by heating slides in either low pH citrate buffer or high pH Tris buffer. The optimal method for antigen retrieval varies for different epitopes, requiring multiple buffers to be used for different targets.

AntiFix™ Universal Antigen Retrieval Buffer has been formulated to provide excellent heat unmasking for a wide variety of targets at neutral pH, reducing the need for buffer optimization. It has been validated in immunofluorescence staining of FFPE tissue with our next-generation fluorescent CF® dyes using HRP-mediated tyramide signal amplification.

Antigen Retrieval Protocol

Note: FFPE sections should be baked onto positively-charged slides to avoid tissue loss during antigen retrieval.

1. Deparaffinize and rehydrate FFPE sections using standard protocols.
2. Place slides in a Coplin jar or in a slide rack inside a microwave-safe container. Note: Microwaving can cause glass jars to crack. Containers should be tested for heat resistance before use.
3. Prepare 1X AntiFix™ Universal Antigen Retrieval Buffer by combining 1 volume of 10X buffer with 9 volumes of dH₂O and mixing completely.
4. Add enough 1X AntiFix™ buffer to completely cover the tissue sections. Usually buffer is added up to the level of the frosted slide labels. Typical volumes are 40 mL for a 5-slide glass Coplin jar or 200 mL for a 24-slide rack staining dish.
5. Heat the slides in the buffer. Two heating methods using a microwave (a) or a multi-cooker (b) are described below. Microwaving is a long-established method for HIER. A kitchen multi-cooker pot with digital temperature control (e.g., Instant Pot®) is a convenient alternative to microwaving. Other heating methods commonly used for HIER (such as a hot water bath or commercial declouing chamber) also can be used.

Note: Kitchen equipment used for tissue processing should be dedicated for research laboratory use, with biohazard labeling as required by your institution or local regulations.

a) Using a microwave: Leave the slide container uncovered or loosely covered to avoid pressure buildup. Microwave the slides on full power until the buffer comes to a boil. Monitoring the container continuously, continue to boil for 10 minutes, stopping the microwave every 30-60 seconds as needed to prevent boil-over of the buffer. After boiling for 10 minutes, allow the container to cool to room temperature.

b) Using a multi-cooker pot: Place the metal steam rack provided with the cooker inside the pot. Leave the slide container loosely covered to avoid pressure buildup. Place the slide container on the rack, making sure it sits securely to avoid tipping. Fill the pot with water so the level comes halfway up the slide container. Close the pot lid and heat at 98°C for 45 minutes without pressure. After heat treatment is complete, carefully remove the slide container from the hot water in the pot and allow to cool to room temperature.

6. Rinse the slides 3 times with dH₂O to completely remove the buffer.
7. Proceed with staining according to your protocol.

Related Products

Catalog number	Product
23007	TrueBlack® Lipofuscin Autofluorescence Quencher
23012	TrueBlack® IF Background Suppressor System (Permeabilizing)
23013	TrueBlack® WB Blocking Buffer Kit
40083	NucSpot® 470 Green Nuclear Counterstain
40061	RedDot™2 Far Red Nuclear Counterstain
23001-23002	EverBrite™ Mounting Medium (with or without DAPI)
23003-23004	EverBrite™ Hardset Mounting Medium (with or without DAPI)
23005	CoverGrip™ Coverslip Sealant
22005	Mini Super ^{HT} Pap Pen 2.5 mm tip, ~400 uses
22006	Super ^{HT} Pap Pen 4 mm tip, ~800 uses
33000 33020	CF® Dye or Biotin Tyramide Amplification Kits
22029	Tyramide Amplification Buffer Plus
92300-92302	Mix-n-Stain™ HRP Antibody Labeling Kits
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
22016	Permeabilization Buffer
22017	Permeabilization and Blocking Buffer
22020	10X Phosphate Buffered Saline (PBS) (4L Cubitainer®)
22013	Bovine Serum Albumin Fraction V
22014	Bovine Serum Albumin 30% solution
22010	10X Fish Gelatin Blocking Agent
22011	Fish Gelatin Powder
30015	DAB Substrate Kit

Please visit our website at www.biotium.com for information on our life science research products, including fluorescent CF® dye primary and secondary antibody conjugates, phalloidins, CF® Dye Tyramide Amplification Kits, Mix-n-Stain™ Antibody Labeling Kits, and other probes and reagents for life science research.

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