Tris-EDTA Antigen Retrieval Solution (50X, pH9.0)

Introduction

Fixation of cells or tissues with paraformaldehyde, formaldehyde, or other aldehyde-based reagents induces cross-linking between proteins, which masks antigenic sites in the sample and leads to reduced staining signals or even false-positive results in immunostaining. Tris-EDTA Antigen Retrieval Solution is one of the most commonly used antigen retrieval buffers. It is used for antigen retrieval in aldehyde-fixed samples such as paraffin-embedded sections and frozen sections, effectively removing aldehyde-induced protein cross-links and fully exposing antigenic epitopes in samples like paraffin sections, thereby greatly improving immunostaining results.

Antigen retrieval is typically required for paraffin sections to enhance immunostaining results. For frozen sections, antigen retrieval is optional; however, many studies have shown that it significantly improves staining outcomes, especially when immunostaining of frozen sections is suboptimal. In principle, for any sample fixed with paraformaldehyde, formaldehyde, or other aldehyde-based reagents—whether frozen sections or cell smears—antigen retrieval effectively removes protein cross-links and fully exposes antigenic epitopes, thereby greatly improving immunostaining results.

This product is a widely used Tris-EDTA buffer (actual pH 9.0 ± 0.2) with an optimized formulation. It is suitable for paraffin sections and can also be used for frozen sections and other sample types.

Protocol

- For paraffin sections

a. Deparaffinization: Immerse sections in xylene for 5 minutes. Replace with fresh xylene and repeat twice (total of 3 xylene washes). Then transfer to absolute ethanol for 5 minutes (twice), then to 90% ethanol for 5 minutes (twice), then to 70% ethanol for 5 minutes (once). Finally, wash with distilled water for 5 minutes (twice).

b. Antigen retrieval: Dilute Tris-EDTA Antigen Retrieval Solution (50X) 50-fold with double-distilled water or Milli-Q water to prepare Tris-EDTA Antigen Retrieval Solution (1X). Immerse sections in Tris-EDTA Antigen Retrieval Solution (1X) and heat at 95–100°C for approximately 15 minutes (heating time can range from 10–20 minutes; the optimal time should be determined empirically based on the sample and target protein). Allow to cool to room temperature for about 20–30 minutes. Wash 1–2 times with Immunostaining Wash Buffer (user-supplied), 3–5 minutes per wash. Proceed with subsequent immunostaining steps such as blocking.

***Note:** Tris-EDTA Antigen Retrieval Solution (1X) should be preheated to 95–100°C before use. Heating can be performed using a conventional water bath or a microwave oven. If using a microwave oven, take care to avoid violent boiling and excessive evaporation.

■ For frozen sections

Wash sections with Immunostaining Wash Buffer (user-supplied) for 5 minutes. Immerse sections in Tris-EDTA Antigen Retrieval Solution (1X) and heat at 95–100°C for approximately 15 minutes (heating time can range from 10–30 minutes; the optimal time should be determined empirically based on the sample and target protein). Tris-EDTA Antigen Retrieval Solution (1X) should be preheated to 95–100°C before use. Allow to cool to room temperature for about 20–30 minutes. Wash 1–2 times with Immunostaining Wash Buffer (user-supplied), 3–5 minutes per wash. Proceed with subsequent immunostaining steps such as blocking.

***Note:** Heating can be performed using a conventional water bath or a microwave oven. If using a microwave oven, take care to avoid violent boiling and excessive evaporation.

■ Other sample types

For antigen retrieval in other sample types, refer to the steps for paraffin sections or frozen sections.

Note

1. Storage conditions: Store at 4°C or -20°C for one year.
2. This product is for scientific research use only.

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