Art.no 60058

Antigen Retrieval Buffer pH 9.0 (10x)

Instructions For Use

For research use only

Manufactured by:

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Intended use

The Navinci Antigen Retrieval Buffer pH 9.0 (10x) is a ready-to-dilute, Tris-EDTA based buffer solution designed for heat-induced epitope retrieval (HIER) on formalin-fixed, paraffin-embedded (FFPE) tissue sections. The buffer is used to unmask antigenic epitopes prior to immunohistochemistry (IHC) staining or *in situ* proximity ligation assay (*is*PLA) workflows.

Principles of the procedure

Antigen retrieval is a critical sample preparation step that restores epitope accessibility in formalin-fixated and paraffin-embedded (FFPE) tissue sections. Poor antigen retrieval can result in weak or absent staining during immunohistochemistry and related techniques such as *is*PLA. Heat-induced epitope retrieval (HIER) is the most widely used and effective method for most antigens. The Antigen Retrieval Buffer pH 9.0 (10x) is specifically formulated for HIER on FFPE tissue sections.

Warnings and precautions

- For research use only. Not for use in diagnostic procedures.
- Not for internal or external use in humans or animals.
- Wear appropriate personal protective equipment (PPE) including lab coat, gloves and protective eyewear when handling reagents.
- Always work in a fume hood when handling xylene and other volatile organic solvents.
- Exercise caution when handling hot solutions and equipment during and after the HIER procedure. Use appropriate heatresistant gloves and handle heated containers with care.
- Unused solutions should be disposed of according to local regulations. The Navinci Antigen Retrieval Buffer pH 9.0 (10x) does not contain hazardous chemicals.

Product content

Storage: 2 to 8°C



Name	Art.no	Amount
Antigen Retrieval Buffer pH 9.0 (10x)	60058	250 mL

General guidelines

- The buffer is supplied as a 10x concentrate. Dilute to 1x working concentration with deionized water before use.
- When stored at 2–8°C, the 10x solution may show signs of precipitation and appear cloudy. Allow the buffer to reach room temperature before use and mix it by inversion 2–3 times before dilution. Ensure that the working solution is well mixed before use.
- Prepare a sufficient volume of 1x working solution to fully submerge all slides during the retrieval procedure.
- For best results use a pressure cooker or dedicated antigen retrieval chamber (e.g., Biocare Medical, Cat. No. DC2012. This helps with the reproducibility of the HIER step.
- Do not reuse the 1x working solution. Discard after each retrieval run.
- Store the 10x concentrate at 2 to 8°C. Do not freeze.
- Do not use the buffer past the expiry date printed on the label.

Required but not provided

Materials

- Incubator (60°C)
- Pressure cooker or decloaking chamber (e.g., Biocare Medical ARC, Cat. No. DC2012)
- Staining jars
- Slide holders/racks
- Tub for slide holder during HIER
- Measuring cylinder
- Timer

Reagents

- Xylene
- Ethanol, 70, 95 and 99.5%
- Tris Buffered Saline (TBS)
- Deionized water

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Instructions for use

1. Preparations

1.1 Remove the Antigen Retrieval Buffer pH 9.0 (10x) from refrigerated storage and allow it to reach room temperature before dilution to ensure that any precipitate that may have formed has dissolved.
1.2 Invert the buffer 2-3 times before proceeding to prepare the 1x working solution (described below).

2. Baking

2.1 Bake the FFPE slides in a pre-heated incubator at 60°C for 45 minutes in a standing (upright) position to allow melted paraffin to drip off the slides.
2.2 Remove the slides from the incubator and allow them to rest at room temperature for 10 minutes before proceeding to deparaffinization.

3. Deparaffinization and rehydration

3.1 Deparaffinize and rehydrate the slides via the xylene-ethanol series shown in Table 1. Move the slides between different staining jars containing the appropriate reagent for the time stated. When lowering the slide holder into the staining jar, move it up and down three times to ensure proper coverage of the sample. After each incubation, lift the slide holder and allow excess reagent to drip off before moving to the next jar.

Table 1. Xylene-ethanol deparaffinization and rehydration series.

Reagent	Time
Xylene	5 minutes
Xylene	5 minutes
Xylene	1 minute
Ethanol 99.5%	3 minutes
Ethanol 99.5%	3 minutes
Ethanol 95%	5 minutes
Ethanol 95%	3 minutes
Ethanol 70%	3 minutes

⚠Caution: Xylene is a hazardous chemical. Always handle xylene in a fume hood and wear appropriate PPE.

3.2 After the final ethanol step, transfer the slides to a staining jar with deionized water. Exchange the water twice for a total of 3 rinsing steps. Keep the slides in water until the antigen retrieval procedure begins.

4. Antigen retrieval (HIER)

4.1 Prepare the 1x working solution by diluting the Antigen Retrieval Buffer pH 9.0 (10x) in Type 1 (deionized) water (Example: 25 mL + 225 mL). Ensure the solution is well mixed.
4.2 Pour a sufficient volume of the 1x retrieval buffer into the slide container (e.g., a slide canister for the pressure cooker). Place the slides into the retrieval buffer, ensuring all tissue sections are fully submerged.
4.3 Place the container with slides and buffer into the pressure cooker or antigen retrieval chamber. Allow the temperature to slowly ramp up from room temperature to 100°C. In the recommended pressure cooker this takes approximately 20 minutes.
4.4 Maintain the temperature at 100°C for 30 minutes.
4.5 After the 30-minute incubation, the temperature will begin to decrease. Allow the temperature to drop to approximately 95°C (this takes only about a minute).
4.6 Carefully remove the slides and the retrieval buffer from the heating device.

⚠Caution: The solution and container will be very hot. Use heat-resistant gloves and exercise caution. Where possible, transfer slides and buffer to a plastic container (rather than metal) to allow for slower, more controlled cooling.

4.7 Leave the slides (still in the retrieval buffer) at room temperature and allow to cool for 30 minutes.

5. Post-retrieval washes

5.1 After cooling, gradually exchange the warm antigen retrieval buffer with deionized water by pouring out half of the antigen retrieval buffer from the staining jar and adding deionized water. Repeat this step twice for a total of 3 2x dilutions with water.
5.2 Exchange the water 4 times.
5.3 Move the slides to a new staining jar with 1x TBS.
5.4 The slides are now ready for the next step of your staining protocol, e.g., applying a hydrophobic border onto the slides, blocking, primary antibody incubation, or a Naveni® assay workflow. For the Naveni® assay workflow, the slides can be kept in 1x TBS in a refrigerator at 2 to 8°C overnight if needed.

6. Disposal

Discard the used 1x antigen retrieval buffer in accordance with local regulations. The Antigen Retrieval Buffer pH 9.0 (10x) does not contain any hazardous chemicals.

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