



Description

5X AntigenPlus Buffer (pH 6)	100 ml	71289-3
5X AntigenPlus Buffer (pH 7.4)	100 ml	71291-3
5X AntigenPlus Buffer (pH 10)	100 ml	71290-3
AntigenPlus Buffer Set		71292-3

For histological studies, chemically fixed tissue sections provide a significant advantage because they can be collected when the biological material is available and saved for later analysis. However, paraformaldehyde or formalin-fixed paraffin embedded tissues sometimes show low reactivity to antibodies used for immunohistochemical staining. Pretreatment of problem tissues with an antigen retrieval buffer can dramatically improve antibody staining. For tissues and antibodies for which antigen retrieval is necessary, Novagen offers 5X AntigenPlus Buffers designed for antigen retrieval applications in fixed, paraffin embedded tissues. There are three different AntigenPlus Buffers available as a 5X concentrates, at pH 6, pH 7.4, and pH 10. 5X AntigenPlus Buffers (pH 6 and pH 10) are used in conjunction with a heat treatment, which has been shown to increase staining intensity with some antigens (1). Treatment with 5X AntigenPlus Buffer (pH 7.4) is performed at room temperature and is a benefit for some fragile tissues(2). Each buffer can be tested with tissues of interest to determine which yields the best result. The AntigenPlus Buffer Set contains 100 ml of each buffer.

Components

AntigenPlus Buffer Set

- 100 ml 5X AntigenPlus Buffer (pH 6)
- 100 ml 5X AntigenPlus Buffer (pH 7.4)
- 100 ml 5X AntigenPlus Buffer (10)

Storage

Store 5X AntigenPlus Buffer (pH 6) and 5X AntigenPlus Buffer (pH 10) at 4°C. Store 5X AntigenPlus Buffer (pH 7.4) at room temperature. If a precipitate forms in the 5X AntigenPlus Buffer (pH 7.4) heat to 37°C for 5–10 min before diluting.



Protocol

The following protocols describe the procedures for using the 5X AntigenPlus Buffers. These protocols are suitable for a range of antigen-antibody combinations, but may require optimization depending on tissue source and antigen-antibody combination. Following treatment, the slides are ready for staining with standard protocols. Negative (tissue known not to react with antibody) and positive (tissues known to react with antibody) controls should be included in the procedures to allow for reliable interpretation of the staining results.

Antigen retrieval using 5X AntigenPlus Buffer (pH 6 or pH 10)

1. Prepare 1X AntigenPlus Buffer by diluting 5X concentrate 1:5 with deionized water.
2. Remove paraffin from tissues by soaking slides 2 times for 10 min in xylene. Use enough volume to cover the slides, typically 100–300 ml.
3. Wash the slides 2 times for 2 min using 100% ethanol, 1 time for 2 min using 95% ethanol, and 1 time for 2 min in 75% ethanol.
4. Wash the slides 2–3 times in deionized water.
5. Place the slides in a Tissue-Tek® Staining Dish (300 ml, VWR Cat. No. 25608-904 or 25608-906) or similar container.
6. Preheat 1X AntigenPlus Buffer (pH 6 or 10) for 5 min in a 600-W microwave on high power.
7. Fill staining dish with preheated AntigenPlus Buffer.

Note: If treating only a few slides, it may be helpful to place blank slides in empty slots to help evenly disperse the heat.

8. Place staining dish with inverted lid in microwave. Microwave on high setting for 20 min. Replace evaporated solution every 5 min and mix solution with a disposable pipet.

Notes: 1X AntigenPlus Buffer should be boiling after 5 min.

If using a smaller container, such as a Coplin Jar, the solution may need to be replenished more frequently.

9. Remove staining dish from microwave and cool at room temperature for 20 min. Do not remove lid during cooling.
10. Wash the slides 2–3 times with room temperature 1X PBS.
11. Proceed with immunohistochemical staining.

Antigen retrieval using 5X AntigenPlus Buffer (pH 7.4)

1. Prepare 1X AntigenPlus Buffer (pH 7.4) by diluting 5X concentrate 1:5 with deionized water.

Note: If a precipitate forms in the 5X AntigenPlus Buffer (pH 7.4) heat to 37°C for 5–10 min before diluting.

2. Remove paraffin from tissues by soaking slides 2 times for 10 min in xylene. Use enough volume to cover the slides, typically 100–300 ml.
3. Wash the slides 2 times for 2 min using 100% ethanol, 1 time for 2 min using 95% ethanol and 1 time for 2 min in 75% ethanol.
4. Wash the slides 2–3 times in deionized water.
5. Place the slides in a Tissue-Tek Staining Dish (300 ml, VWR Cat. No. 25608-904 or 25608-906) or similar container.
6. Fill staining dish with room temperature 1X AntigenPlus Buffer (pH 7.4). Incubate at room temperature for 5–10 min.
7. Wash the slides 2–3 times with room temperature 1X PBS.
8. Proceed with immunohistochemical staining.



References

1. Evers, P. and Uylings, H. B. (1994) *J. Histochem. Cytochem.* **42**, 1555–1563.
2. Subbotin, A. and Handley, M. (2003) *inNovations* **17**, 17–18.