2505 Parview Road • Middleton, WI 53562-2579 • 800-383-7799 • www.newcomersupply.com • newly@newcomersupply.com

February 2016

Buffer Solutions for Epitope Retrieval- Technical Memo

SOLUTIONS:	500 ml	1 Liter	1 Gallon
EDTA Buffer 0.001M, pH 8.0	Part 1056A	Part 1056B	Part 1056C
Citrate Buffer 0.01M, pH 6.0	Part 10355A	Part 10355B	Part 10355C

For storage requirements and expiration date refer to individual bottle labels.

APPLICATION:

Newcomer Supply Buffer Solutions for Epitope Retrieval procedure provides a choice of two ready-to-use buffers for antigen retrieval. The majority of epitopes/antigens are masked in formalin fixed paraffin embedded (FFPE) tissues. Antigen retrieval methods improve antibody binding by de-masking the FFPE chemical modification of epitopes through heat induced epitope retrieval (HIER) procedures when performed prior to immunohistochemical (IHC) staining.

No retrieval buffer is optimal for all tissue antigens. The choice of one buffer over another will depend upon the suggested retrieval buffer specific to an individual antibody. Refer to each antibody datasheet for recommended chemical composition and pH value of retrieval buffer.

- EDTA Buffer 0.001M, pH 8.0 is an alkaline buffer optimal for use with primary antibodies that require an EDTA buffer at a higher pH for HIER.
- Citrate Buffer 0.01M, pH 6.0 is an acidic buffer optimal for use with primary antibodies that require a citrate buffer at a lower pH for HIER.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections on adhesive slides
Solutions: All solutions are manufactured by Newcomer Supply, Inc.

EPITOPE RETRIEVAL PROCEDURE:

1. Choose a method of HIER that best suits the laboratory environment and anticipated work load.
    a. *Instrumentation and methods for HIER include but not limited to: microwave, pressure cooker and steamer methods.*
2. To validate instrumentation: follow manufacturers suggested instructions for antigen retrieval methods. Successful HIER is dependent on temperature, time, pH and chemical composition of the buffer. Some variations in suggested instructions may be required for optimal results.
    a. *See Procedure Note #1.*
3. After validation of retrieval instrumentation and methodology; deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
    a. *See Procedure Notes #2 and #3.*
4. Proceed with a validated method of HIER per established laboratory protocol implementing either EDTA Buffer 0.001M, pH 8.0 (1056) or Citrate Buffer 0.01M, pH 6.0 (10355) according to antibody recommendations.
5. After completion of HIER, allow sufficient time for slides to cool before proceeding with IHC protocol.

PROCEDURE NOTES:

1. The specific tissue type, fixative, section thickness and/or primary antibody used are additional factors for consideration in the retrieval validation process.
2. Drain staining rack/slides after each step to prevent solution carry over.
3. Do not allow sections to dry out at any point during staining procedure.
4. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 442-445, 458-459.
2. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 281-282.
3. Shi, Shan-Rong, Richard J. Cote, Lillian L. Young, and Clive R. Taylor. "Antigen Retrieval Immunohistochemistry: Practice and Development." *The Journal of Histotechnology* 20.2 (1997): 145-154.
4. Tacha, David, and Maria Teixeira. "History and Overview of Antigen Retrieval: Methodologies and Critical Aspects." *The Journal of Histotechnology* 25.4 (2002): 237-242.
5. Taylor, Clive R., Shan-Rong Shi, Chen Chen, Lillian Young, Christina Yang, and Richard J. Cote. "Comparative Study of Antigen Retrieval Heating Methods". *CAP Today*, September 1995, 16-22.
6. Modifications developed by Newcomer Supply Laboratory.