

L.A.B. Solution (Liberate Antibody Binding Solution)

Catalog Number 24310

Introduction:

Exposing primary antigen binding sites for immunohistochemistry (IHC) in formalin fixed paraffin embedded (FFPE) tissue is one of the most difficult problems in Histology today. L.A.B. Solution liberates the antigenic sites allowing primary antibody binding and subsequent detection. Heat is not necessary in most cases to liberate the antigenic site and incubation times with L.A.B. Solution range from five to twenty minutes. Carrying the reaction out at room temperature enables the use of L.A.B. Solution with automated stainers as well as by manual procedures.

The development of L.A.B. Solution resulted from conversations among Polysciences' scientists familiar with DNA protein interactions and Histology techniques who were interested in researching tumor origins in archived paraffin embedded tissues. These conversations resulted in improved methods for exposing epitopes for staining including reducing the time and heat required by other methods. The need to expose cross-linked or denatured proteins caused by fixation is critical for staining and identification in protein sequencing. L.A.B. Solution provides a convenient way to expose cross-linked and/or denatured proteins, creating better conditions for reacting tissue sections.

L.A.B. Solution can be used at room temperature, enabling the liberation of antibody binding sites without the use of heat. Incubating L.A.B. Solution with Estrogen Receptor or Progesterone Receptor antibodies for five to ten minutes at room temperature results in excellent staining. This avoids any heating that may cause sections to loosen and fold over or fall off the slide. Using L.A.B. Solution can also result in significant time savings. Current protocols may consist of preheating solutions for 5 to 10 minutes to 95-100°C. Slides are added to the heated solution and incubated for 20 minutes, followed by cooling for 20 minutes. The 5 to 20 minutes room temperature incubation with L.A.B. Solution results in a time savings of at least 35 minutes from the start of the protocol to getting the slides on the pathologist desk for review. Obtain the same time savings with many other antibodies. Some antibody binding sites may still require heating for effective results. To liberate binding sites for more difficult antibodies, L.A.B. Solution should be heated to 60°C in a conventional oven. The slides can then be added to the heated L.A.B. Solution and incubated for 5 to 20 minutes in a 60°C oven, followed by IHC staining. No cool down period is required prior to IHC procedures. See Table 1 for a list of examples for other antibodies.

Advantages of L.A.B. Solution

- Eliminate or reduce the use of heat and protect tissue sections
- Shorten overall staining time
- Use directly on automated immuno stainers
- No special equipment required

Requirements for Use of L.A.B. Solution:

Tissue must be well fixed in 10% Neutral Buffered Formalin or other Formaldehyde/Paraformaldehyde based fixative. As with any protocol used to liberate antigenic binding sites, it is important to fix the tissue evenly throughout the block. Specimens with only the outer surface well fixed and the interior layers lightly fixed may result in staining confined to either the edges of the section or the center of the section. The result may be light to negative staining on the internal portion of the specimen. Standard processing for routine paraffin specimens is the recommended procedure.

Slides must be deparaffinized by standard laboratory protocol prior to beginning this procedure.

Procedures for Use of L.A.B. Solution

The time required for antigen liberation may vary with laboratory requirements, antibody dilutions, and various antibodies and detection systems. It is always recommended that control tissue be used to assess the time required for each antibody incubated with L.A.B. Solution or any new protocol for clinical or research work.

Procedure for Using L.A.B. Solution with Automated Immuno Stainers:

1. Automated Immuno Stainers will require a step to be programmed in at the beginning of the standard staining run.
2. The volume of L.A.B. Solution used will depend on the size of the section being stained. The time required to complete the antigen/epitope liberation step is 5 to 20 minutes at room temperature followed by a buffer rinse.
3. The standard program for incubation with the antibodies and detection remains unchanged.
4. Refer to your instrument manual for making any programming changes.

Manual Procedure for Using L.A.B. Solution:

1. When manually staining large volumes of slides, place the slides in a Coplin jar or slide rack in a dish and fill with L.A.B. Solution until sections are covered.
2. Incubate for 5 to 20 minutes at room temperature to liberate the antigen/epitope site.
3. Slides can now be loaded on an Automated Immuno Stainer for using the standard staining program.
4. For a small number of slides, pipette L.A.B. Solution directly onto the section area until covered. L.A.B. solution will not dissolve barriers created with PAP Pens.

Note: To liberate more difficult antigen/epitope sites that require heat, preheat L.A.B. Solution to 60°C in a standard laboratory oven. Place the slides in the preheated L.A.B. Solution, then in a 60°C oven for 5 to 20 minutes. No cool down is required. Briefly rinse the slides in deionized water, PBS, or other buffer of choice. The slides can be moved directly to the next step of the staining procedure whether manual or automated. This procedure allows the laboratory to use readily available equipment and prevents the exposure of laboratory personnel to very hot fluid transfers. L.A.B. Solution can be kept tightly closed in a 60°C oven for one week and reused as needed.

Storage of L.A.B. Solution

L.A.B. Solution is stored at room temperature. It should not be refrigerated or stored in direct sunlight.

When working with more difficult antigen/epitope sites that require heat, L.A.B. solution can be preheated to 60°C and kept tightly closed in a 60°C oven for one week and reused as needed.

Safety and Handling Precautions:

L.A.B. Solution is harmful if swallowed. Drink copious amounts of water and call a physician if it is ingested accidentally. May cause skin and eye irritation.

Table 1: Typical Protocols for Exposing Antigen Binding Sites Using L.A.B. Solution Compared to Standard HIER (Heat Induced Epitope Retrieval) Methods

Antibody	L.A.B. Solution Treatment	Standard HIER Treatment
Estrogen Receptor	5 minutes at Room Temperature	20 min. incubation in Citrate Buffer at 100°C with 20min. Cool Down
Progesterone Receptor	5 minutes at Room Temperature	20 min. incubation in Citrate Buffer at 100°C with 20min. Cool Down
Synaptophysin	5 minutes at Room Temperature	20 min. incubation in 2X Citrate Buffer at 100°C with 20 min. Cool Down
Chromogranin	5 minutes at Room Temperature	20 min. incubation in 2X Citrate Buffer at 100°C with 20 min. Cool Down
CK 5/6	5 minutes at 60°C with no Cool Down	20 min. incubation in 2X Citrate Buffer at 100°C with 20 min. Cool Down
TAU	15 minutes at 60°C with no Cool Down	20 min. incubation in Citrate Buffer at 100°C with 20min. Cool Down
Epstein Barr Virus EBV	5 minutes at Room Temperature	20 min. incubation in 2X Citrate Buffer at 100°C with 20 min. Cool Down
CMV	5 minutes at Room Temperature	5 minute incubation in Protease K at 100°C
Vimentin	5 minutes at Room Temperature	20 min. incubation in 2X Citrate Buffer at 100°C with 20 min. Cool Down
BCL-1	10 to 15 minutes at Room Temperature	20 min. incubation in Borg at 100°C with 20 minute Cool Down
S-100	5 minutes at Room Temperature	20 min. incubation in 2X Citrate Buffer at 100°C with 20 min. Cool Down
CD30	5 Minutes at 60°C with no Cool Down or 15 minutes at Room Temperature	20 min. incubation in 2X Citrate Buffer at 100°C with 20 min. Cool Down
LCA (CD45)	10 Minutes at Room Temperature	5 minute incubation in Protease K at room temperature
All CD Markers work best in well fixed tissues	5 to 10 minutes at Room Temperature	Various methods of HIER are used and Digestion as listed in Manufacturer's Antibody Data Sheet

Ordering Information:

Catalog #	Description	Size
24310-500	Liberate Antibody Binding (L.A.B.) Solution	500ml

To Order:

In The U.S. Call: 1-800-523-2575 • 215-343-6484
 In The U.S. FAX: 1-800-343-3291 • 215-343-0214
 In Germany Call: (49) 6221-765767
 In Germany FAX: (49) 6221-764620

Order online anytime at www.polysciences.com

For our full line of Histology Products, request a Catalog by calling or visit our web site at www.polysciences.com.

Should any of our materials fail to perform to our specifications, we will be pleased to provide replacements or return the purchase price. We solicit your inquiries concerning all needs for life sciences work. The information given in this bulletin is to the best of our knowledge accurate, but no warranty is expressed or implied. It is the user's responsibility to determine the suitability for his own use of the products described herein, and since conditions of use are beyond our control, we disclaim all liability with respect to the use of any material supplied by us. Nothing contained herein shall be construed as a recommendation to use any product or to practice any process in violation of any law or any government regulation.