

The Basic Truth:
Antigen Retrieval Solutions and pH

The Basic Truth: Antigen Retrieval Solutions and pH

Most immunohistochemistry (IHC) specimens are routinely fixed in formalin and, while formalin provides excellent preservation of morphology, it can greatly diminish the sensitivity to IHC. Formalin creates cross-links that alter protein biochemistry and can block antigenic sites. Antigen retrieval will be needed to break these cross-links and expose the antigen's epitope.

The antigen retrieval process has many variables, including the target antigen, the antibody, fixation time, tissue processing and the antigen retrieval method - heat-induced or enzymatic. For heat-induced epitope retrieval (HIER), pH and composition of your retrieval solutions are important factors. HIER solutions can generally be grouped into three categories based on pH and composition:

pH Categories

1. Low pH (~3-5) buffered frequently by glycine-HCl
2. Slightly low to neutral pH (~6-7) buffered with citric acid
3. High pH (~8-10) buffered with EDTA or Tris

While citrate buffers (pH ~6) are used often as they nicely preserve tissue morphology, alkaline solutions (pH ~8-10) tend to increase staining intensity for most antibodies. EDTA buffers are recommended for over fixed tissue or antigens that are hard to detect. However, higher pH and EDTA based solutions do have drawbacks, which can include loss of sections from the microscope slide and distorted morphology.

Most antibodies have antigen retrieval solution recommendations on their datasheet; however, there are times when the best solution must be found experimentally. Since there is no universal retrieval buffer, researchers and technicians may need to test a variety of buffers with varying composition and pH, along with different retrieval times and temperatures to see which gives optimal staining.

Biocare Medical offers a full range of antigen retrieval solutions. These “Decloaking” solutions are available in Citrate, Tris and EDTA bases with a pH between 6.0 and 9.5. All of Biocare’s Decloaker solutions incorporate Assure™ color-coded pH indicator technology, which assures by visual inspection that the solution is at the correct dilution and pH. These buffers are specially formulated for superior pH stability at high temperatures and will help prevent the loss of pH-sensitive antigens.

Biocare Medical Antigen Retrieval Solutions

HIER Buffers	Status	Buffer Base	pH	Surfactant	Usage	Formulation	Volume	Cat. No.
Diva Decloaker	IVD	Citrate	pH 6.2	Yes	IHC	Ready-to-Use	1000 mL, 1 G	DV2004 MM, G1
Diva Decloaker	IVD	Citrate	pH 6.2	Yes	IHC	Concentrate, 10X	100, 500 mL	DV2004 LX, MX
Diva Decloaker	IVD	Citrate	pH 6.2	Yes	IHC	Concentrate, 20X	250 mL	DV2005 L2J
Reveal Decloaker	IVD	Citrate	pH 6.0	Yes	IHC, ISH	Ready-to-Use	1000 mL, 1 G	RV1000 MMRTU, G1
Reveal Decloaker	IVD	Citrate	pH 6.0	Yes	IHC, ISH	Concentrate, 10X	500 mL	RV1000 M
Borg Decloaker	IVD	Tris	pH 9.5	Yes	IHC	Ready-to-Use	250, 1000mL, 1G	BD1000 S-250, MM, G1
EDTA Decloaker	IVD	EDTA	pH 8.5	No	IHC	Concentrate, 5X	100, 500 mL	CB917 L, M
Rodent Decloaker	RUO	Citrate	pH 6.2	Yes	Rodent IHC	Concentrate, 10X	100, 500 mL	RD913 L, M
RISH™ Retrieval	IVD	Citrate	pH 6.2	Yes	ISH	Concentrate, 10X	500 mL	RI0209 M
Antigen Decloaker	IVD	Citrate	pH 6.0	No	IHC	Concentrate, 10X	500 mL	CB910 M
Nuclear Decloaker	IVD	Tris	pH 9.5	No	IHC	Concentrate, 10X	500 mL	CB911 M

To learn more about Biocare Medical’s antigen retrieval buffers and devices, please call anytime at 800-799-9499 or visit our website: <https://biocare.net/products/ancillaries/antigen-retrieval-solutions/>