WHITEPAPER

A Rev-elution-ary Solution to Eliminate Cross-Reactivity!

BIOCARE MEDICAL

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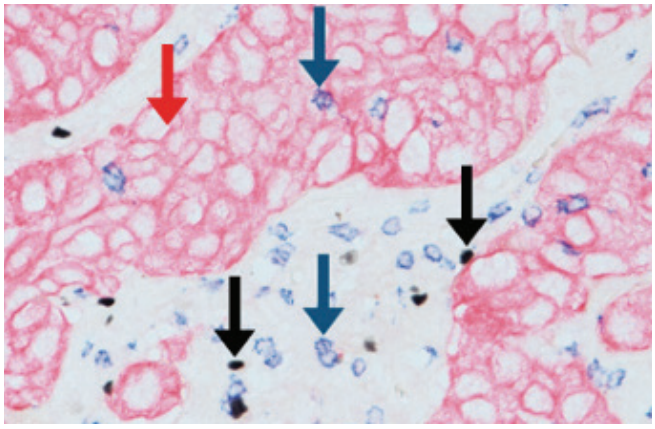
How to Utilize a Denaturing Solution Step to Eliminate Cross-Reactivity

Double or multiple antigen labeling in immunohistochemistry (IHC) classically relies on the existence of primary antibodies raised in different species or of different IgG isotypes to ensure the specific labeling with the secondary detection systems.[1] With an abundance of mouse primary antibodies and increase of rabbit primary antibodies, it is easier than ever to conduct chromogenic multiplex (IHC) staining. However, not infrequently, the best-suited antibodies for a study are only available as the same IgG isotype of the same species, and the scarcity of the antigen makes a direct labeling approach impractical. In order to limit cross-reactivity, an elution step is required.

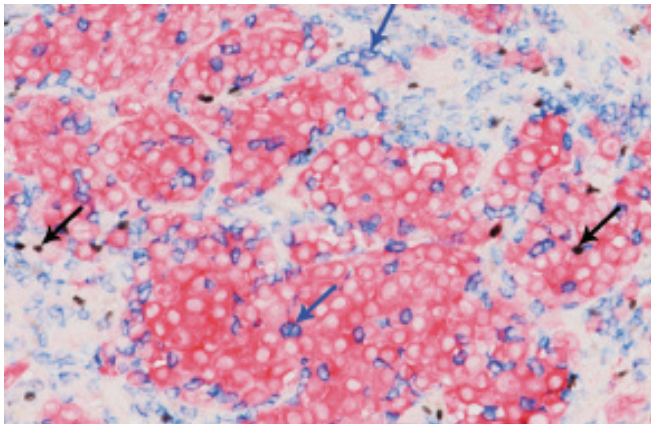
Biocare's Denaturing Solution can be used as an elution step when performing a sequential double stain and/or multiplex technique. This solution "serves to remove previously bound primary and link antibodies leaving only the deposit of chromogen from the previous steps, thus eliminating any potential for cross-reactivity."[2] The solution is supplied as 2 separate components that are mixed at different dilutions depending on the antigen retrieval method used (i.e. no retrieval, enzyme digestion, high pH or low pH retrieval solutions).

Get Started: How to Implement Denaturing Solution in Your Protocol

To use, simply deparaffinize tissue and hydrate to water. Digest or retrieve the tissue and block for endogenous peroxidase and non-specific protein, if desired. Apply the first primary antibody, followed by the appropriate secondary antibody and chromogen (recommended to use the most "robust" chromogen dyes (such as DAB, AEC, etc.), followed by less "robust' chromogens (Fast Red, etc.)). Then employ the Denaturing Solution for 2-5 minutes. After rinsing with buffer, the second primary antibody can be dispensed, followed by the corresponding secondary and chromogen. With the assurance of no cross reactivity, the slides are ready to be counterstained, dehydrated, and mounted. Let your Rev-'Elution' begin!



A triple stain of poorly differentiated colon adenocarcinoma showing CD8 + (blue arrows), FOXP3 + (black arrows), and CK8/18 + colon adenocarcinoma (red arrows). A cocktail of FOXP3 (mouse monoclonal) + CD8 (rabbit monoclonal) was developed first, followed by an elution step using Denaturing Solution, and then CK8/18 (rabbit monoclonal) development.[3]



A triple stain utilizing Denaturing Solution: Melanoma TILs stained with a mouse monoclonal FOXP3+ (black, black arrows), rabbit monoclonal CD8 + (blue, blue arrows) and a mouse monoclonal PMC-2 (red mask), 20x.[4]

To learn more about the Biocare's Denaturing Solution or other dual stain reagents, please contact us anytime at 800-799-9499 or check out the following links: www.biocare.net and https://biocare.net/product/denaturing-solution-elution-step/

1. Pirici D, *et al.* Antibody Elution Method for Multiple Immunohistochemistry on Primary Antibodies Raised in the Same Species and of the Same Subtype J Histochem Cytochem. 2009 Jun; 57(6): 567–575. 2. Handbook: Immunochemical Staining Methods- 3rd Edition, 1 January 2001, http://www.ihcworld.com/_books/Dako_Handbook.pdf 3. Yuan, W, *et al.* Immunohistochemical Double and Triple Stain Strategies with CD8, CD103, FOXP3, PD-1 and CK8/18 in Colon Adenocarcinoma: Quantitation, Prognosis and Immunotherapy, March 2016, https://biocare.net/wp-content/uploads/SP-DBLTRPL-100.pdf 4. Tacha, D, *et al.* Immunohistochemical multiplex staining strategies with CD8, CD103, PD-1, FOXP3 and pan melanoma cocktail in tumor infiltrating lymphocytes in melanoma, April 2016, https://biocare.net/wp-content/uploads/SP-MPXSTRAT100.pdf