DOWNLOAD PDF ANTIGEN RETRIEVAL

Chapter 1 : TintoRetriever Pressure Cooker - Bio SB

Most formalin-fixed tissues require an antigen retrieval step before immunohistochemical staining. Methylene bridges formed during fixation cross-link proteins and mask antigenic sites. Antigen retrieval methods break these methylene bridges and expose antigenic sites, allowing antibodies to bind.

First you load the slides into Slide Rack. This means you can use up to 6 different buffers at once. This is an affordable solution to all known major problems with immunohistochemistry on paraffin sections. Ease of use combined with high reproducibility of the results will give you the best quality immunostaining. The Retriever is a bench-top model for thermally processing slides of formalin-fixed, paraffin embedded tissues prior to immunostaining. The Retriever preserves processed tissues. This is very important when you are trying to find the most appropriate buffer for epitope recovery or need to process the same tissue in different buffers for different antibodies. This is especially critical for retrospective research that involves IHC on large collections of tissues that may not be all available at once. Load the sections, pour the buffer, close the lid and push the button. No training, not "tricks of the trade", no waiting when the diagnostic pathology has time to do it for you. Use either standard Citrate of EDTA buffers, or our buffers new Universal set of buffers arrives this fall Not to worry that the machine will stop working. This only happened twice in while history of Retriever, and both times it was the result of improper exploitation. How does the Retriever work? It looks as a pressure cooker, and basically it is a pressure cooker. People ask us sometimes what temperature and pressure are used to imitate it with some other pressure cooker. However, this information will not be helpful, because the key factor is not what temperature and pressure it reaches, but how: We did a lot of tests to find this optimal setting. When the required temperature is reached, it will be kept for several minutes. After that the slides will be cooling over 2 hours. Specially designed thermal walls of the unit control the speed of cooling of the inner chamber and slides. This is an important stage of the process as well: Who would benefit from using our Retriever? Investigative Pathology, where the high quality of staining a picture may be published! Safety and Quick-Start Guide Hard plastified copies of these are always supplied with Retriever, however, if you would like to just get acquainted with the procedure, or replace the ones you had, please download them in. For most antibody stainings the slides can be used immediately. A reliable, time tested solution to unmask antigen on formalin-fixed sections.

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Chapter 2 : Antigen retrieval - OpenWetWare

Antigen Retrieval Techniques The need for antigen retrieval depends on multiple variables, including but not limited to, the target antigen, the antibody used, the type of tissue, and the method and duration of fixation.

All protocols Epitope Retrieval Why is antigen retrieval necessary? The impaired ability of antibodies to access epitope in fixed tissue can impact IHC staining. Protein fixation creates crosslinks that mask tissue antigen and limit antibody-epitope binding. In addition to changes in epitope conformation and electrostatic charge, masking is the result of crosslinks created between amino acids within the target antigen and between proteins surrounding the target antigen. What is antigen retrieval and how does it work? Antigen retrieval refers to any method which restores antigenicity and enhances antibody-epitope binding by reversing protein crosslinks in tissue sections. When should I run an antigen retrieval step? Antigen retrieval is recommended for most tissue antigens following deparaffinization and rehydration of formalin-fixed tissues in the immunohistochemistry staining protocol. Is antigen retrieval necessary on frozen tissue sections? Antigen retrieval on frozen tissue is not recommended. The retrieval process can be too harsh and damage the tissue. However, it is often recommended to restore antigenicity in formalin-fixed tissues. Is antigen retrieval always required on formalin-fixed paraffin-embedded tissues? Not all IHC experiments require a retrieval step. The fixation method and length, in addition to the type of tissue, target antigen, and antibody determine the need for antigen retrieval. For instance, without an antigen retrieval step, a polyclonal antibody may enhance detection of antigen compared to a monoclonal due to its ability to bind multiple epitopes. A change in pH or cation concentration of antibody diluent or a simple change in the incubation conditions of primary antibody can also improve antibody affinity for its epitope without the need for a formal antigen retrieval step. However, antigen retrieval is recommended for most formalin-fixed paraffin-embedded tissues. What is the difference between heat-induced epitope retrieval and proteolytic-induced epitope retrieval? One of two antigen retrieval methods is recommended on most formalin-fixed paraffin-embedded tissue sections. Proteolytic-Induced Epitope Retrieval PIER is an enzymatic method of antigen retrieval which relies on enzymes such as proteinase K, trypsin, or pepsin to unmask antigen. Because ideal retrieval conditions are influenced by the tissue type and fixation method, optimization of the retrieval protocol for each antigen is highly recommended.

Chapter 3 : Antigen Retrieval Protocol: Novus Biologicals

As a review for the 20th anniversary of publishing the antigen retrieval (AR) technique in this journal, the authors intend briefly to summarize developments in AR-immunohistochemistry (IHC)-based research and diagnostics, with particular emphasis on current challenges and future research.

Fixation can alter protein biochemistry such that the epitope of interest is masked and can no longer bind to the primary antibody. Masking of the epitope can be caused by cross-linking of amino acids within the epitope, cross-linking unrelated peptides at or near an epitope, altering the conformation of an epitope, or altering the electrostatic charge of the antigen. Antigen retrieval refers to any technique in which the masking of an epitope is reversed and epitope-antibody binding is restored. Antigen Retrieval Techniques The need for antigen retrieval depends on multiple variables, including but not limited to, the target antigen, the antibody used, the type of tissue, and the method and duration of fixation. Because they recognize multiple epitopes, polyclonal antibodies are more likely than monoclonal antibodies to detect a given antigen without the use of antigen retrieval techniques. There are multiple techniques to restore the immunoreactivity of an epitope. Methods as simple as changing the pH or the cation concentration of the antibody diluent can influence the affinity of an antibody for its epitope. For partially masked epitopes it may be appropriate to first try increasing the primary antibody incubation conditions before commencing antigen retrieval. However, this step will also require further optimization in terms of appropriate antibody concentration, incubation time, and temperature. When discussing antigen retrieval methods, techniques generally fall into two main categories, protease-induced epitope retrieval PIER and heat-induced epitope retrieval HIER. Once optimized, the effects of antigen retrieval can be pronounced. The mechanism of action is thought to be the cleavage of peptides that may be masking the epitope. The disadvantages of PIER are the low success rate for restoring immunoreactivity and the potential for destroying both tissue morphology and the antigen of interest. The protocol must be optimized for each tissue, fixation method, and antigen to be studied. HIER is performed using microwave ovens, pressure cookers, vegetable steamers, autoclaves, or water baths. Microwaves are an increasingly popular appliance for HIER. These protocols tend to involve 5 minute periods of heat followed by replacement of the buffer. HIER is especially time-, temperature-, buffer-, and pH-sensitive, and the best method must be determined empirically. In addition, the technique is often too harsh for cryostat tissue sections and alcohol-fixed tissue. The time, temperature, and pH must also be optimized for each appliance i. To optimize antigen retrieval preliminary studies must be conducted using a matrix of time, temperature, and pH combinations. The possibility of artifactual staining should always be considered when using any antigen retrieval methodology. The use of controls to demonstrate specific antibody binding should be included since staining is influenced by multiple variables in any given experiment. During initial optimization, investigators can compare samples treated with neutral pH 7. Subsequent tests using acidic or basic antigen retrieval solutions may be necessary depending on the tissue. Acidic and basic antigen retrieval solutions are more likely to affect tissue morphology than a neutral solution.

Chapter 4 : IHC antigen retrieval protocol | Abcam

Antigen retrieval enables an antibody to access the target protein within the tissue. Masked epitopes can be recovered using either enzymatic/proteolytic antigen retrieval, or heat-induced antigen retrieval methods.

Most formalin-fixed tissues require an antigen retrieval step before immunohistochemical staining. Methylene bridges formed during fixation cross-link proteins and mask antigenic sites. Antigen retrieval methods break these methylene bridges and expose antigenic sites, allowing antibodies to bind. The two methods for antigen retrieval are heat induced epitope retrieval HIER and enzymatic retrieval. Enzymatic retrieval can sometimes damage the morphology of the section, so the concentration and treatment time need to be tested. Heat-induced epitope retrieval is most often performed using a pressure cooker, a microwave, or a vegetable steamer. This is useful when working with tissue sections that fall off the slide when heated at higher temperatures; in particular bone, cartilage, and skin. Unless the antigen retrieval method is stated on the antibody datasheet, the optimal method for each antigen must be found experimentally. This applies also to the choice of buffer used for heat-mediated retrieval. We recommend testing several methods to find the retrieval method that gives optimal staining. Convenient buffers for confident results For robust results with heat-mediated antigen retrieval, we recommend our pre-formulated antigen retrieval buffers. We also offer a Universal Heat-mediated Antigen Retrieval Reagent kit used with our leading PD-L1 clone that is compatible with most antibodies and removes the need for multiple buffers. For enzymatic antigen retrieval, we recommend our Trypsin solution kit. We also offer a Pepsin solution kit and Proteinase K solution kit. Alternatively, you can prepare your own buffers and solutions and use the same recommended methods below. In the absence of datasheet information, choice of retrieval buffer is best accomplished by trial. Sodium citrate buffer 10 mM Sodium citrate, 0. Adjust pH to 6. Adjust pH to 9. Heat-induced epitope retrieval methods: Add the appropriate antigen retrieval buffer to the pressure cooker. Place the pressure cooker on the hotplate and turn it on full power. Do not secure the lid of the pressure cooker at this point, simply rest it on top. While waiting for the pressure cooker to come to a boil, de-paraffinize and rehydrate the sections. Once boiling, transfer the slides from the tap water to the pressure cooker. Use care with hot solution â€" use forceps. As soon as the cooker has reached full pressure, time 3 min. When 3 min have elapsed, turn off the hotplate and place the pressure cooker in an empty sink. Activate the pressure release valve and run cold water over the cooker. Once de-pressurized, open the lid and run cold water into the cooker for 10 min. This is to allow the slides to cool enough so they may be handled, and to allow the antigenic site to re-form after being exposed to high temperature. Handle hot solutions with care. Continue with the immunohistochemical staining protocol. Hot and cold spots are common, leading to uneven antigen retrieval. Antigen retrieval times are usually longer, due to the absence of a pressurized environment, nearly always leading to section dissociation. A scientific microwave is more appropriate. When using this method, it is possible for the buffer to boil over, and a large amount of the retrieval buffer will evaporate. Be sure to watch the buffer level of the slide vessel, and add more buffer if necessary. Do not allow the slides to dry out. Slides should be placed in a plastic rack and vessel for this procedure. Standard glass histology staining racks and vessels will crack when heated. Method Deparaffinize and rehydrate the sections. Add the appropriate antigen retrieval buffer to the microwaveable vessel. Use a non-sealed vessel to allow for evaporation during the boil. Be sure to monitor for evaporation and watch out for boiling over during the procedure and do not allow the slides to dry out. Place the slides in the microwaveable vessel. Place the vessel inside the microwave. If using a domestic microwave, set to full power and wait until the solution comes to the boil. Boil for 20 min from this point. When 20 min has elapsed, remove the vessel and run cold tap water into it for 10 min. Use care with hot solution. This allows the slides to cool enough so they may be handled, and allows the antigenic site to re-form after being exposed to high temperature. Slides should be placed in a plastic or metal rack and vessel for this procedure. Pre-heat the appropriate antigen retrieval buffer to boiling in a flask. Put the container that will hold the rack of slides into the vegetable steamer. Carefully add the hot buffer to the container, followed by the rack of slides. If more convenient, add the buffer to the container before placing the container in the steamer. Close

the lid of the steamer. The container of buffer should also have a lid. Keep the container in the steamer for 20 min from this point. There are at least two methods for applying the enzyme solution to the tissue: The first method uses less reagent but since each slide needs to be handled individually, the incubation time needs to be monitored carefully for each slide to ensure all slides are receiving the same treatment. For this reason, it is easier to treat large batches of slides by immersing them in a container of enzyme solution. If using an automated staining system eg Ventana , consult the manufacturer for an appropriate enzymatic retrieval protocol. It may be necessary to spread the solution around the section with the pipette tip; be careful not to damage the tissue. Avoid placing the slides directly on the incubator shelves as there will be variations in temperature that could affect staining intensity. Ideally, the container holding the slides is pre-heated in the incubator. After 10â€”20 min this will need to be optimized , remove the slides from the incubator and transfer to a rack in a container of tap water. Rinse by running tap water for 3 min. Continue with immunohistochemical staining protocol. Set water bath to the optimal temperature for the enzyme you are using. Add ultrapure water to two containers that can hold slide racks. Place the containers into the water bath to warm. Use a sufficient volume of water or buffer to cover the slides. Deparaffinize and rehydrate sections as above. Place slides in one water container to warm. Placing cold slides into the enzyme solution will lower the temperature of the solution, reducing enzyme activity and leading to under-retrieval of the antigenic site. Prepare the enzymatic antigen retrieval buffer from the warm water in the other container, and then return the container to the water bath to allow the solution to re-heat. Prepare the enzymatic antigen retrieval solution as quickly as possible to avoid impairing the activity of the enzyme. Allow this solution to return to temperature before introducing the slides. Transfer the warmed slides into the enzyme solution for 10â€”20 min with intermittent gentle agitation, then remove the slides and place them in running tap water for 3 min to rinse off the enzyme. Retrieval time should be optimized by incubating the tissue section in the enzyme solution for 10, 15, 20, 25 and 30 min before immunohistochemical staining. You can also access our most popular protocols straight from your phone with the Abcam app, which features protocols, scientific support and a suite of useful tools that are handy for any bench scientist. Get resources and offers direct to your inbox Sign up A-Z by research area.

Chapter 5 : A simple and effective heat induced antigen retrieval method

Antigen retrieval is an approach to reducing or eliminating these chemical modifications. The two primary methods of antigen retrieval are heat-mediated epitope retrieval (HIER) and proteolytic induced epitope retrieval (PIER).

Chapter 6 : IHC-F Protocol | IHC Protocol | Immunostaining

Which antigen retrieval method should I use for a specific antibody Some companies test their antibodies for immunohistochemistry application and will include a suggested antigen retrieval method in the datasheet (note: some of the suggested method is not optimal).

Chapter 7 : Antigen Retrieval Technical Tips

antigen retrieval A process in which tissues are heated with a microwave oven, steam (pressure) cooker, autoclave or ultrasound, which causes antigens that are difficult or impossible to stain in formalin-fixed paraffin sections of tissue to become readily stainable by standard immunohistochemical methods.

Chapter 8 : Antigen retrieval for IHC | Abcam

Antigen Retrieval Protocols The demonstration of many antigens can be significantly improved by the pretreatment with the antigen retrieval reagents that break the protein cross-links formed by formalin fixation and thereby uncover hidden antigenic sites.

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Chapter 9 : Antigen retrieval - Wikipedia

In many cases the fixation and processing steps involved in the preparation of tissue results in loss of antigen immunoreactivity. Often this can be reversed by using appropriate antigen retrieval techniques such as microwave antigen retrieval or proteolytic digestion. Standard protocols for some of these techniques are outlined below.