



The Anatomy of a Primary Antibody Data Sheet

Just what does all of it mean???

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So your pathologist wants you to order and work up a new primary antibody with your routine immunohistochemical procedure. Of course you have never heard of the antibody and have no experience with using it, so where do you start? How do you figure out the recommended dilution? What positive control can you use? How long of an incubation time should you try? What type of detection system will get this antibody to work on your tissue? Do you need any type of epitope retrieval, either heat-induced or enzyme-based treatments? If so, what concentration or pH does the solution need to be? And for how long should it be treated? And at what temperature?

There is one place to begin, that should be provided to you along with the antibody. This is the antibody technical data sheet. This sheet (or sheets) of paper holds a ton of information for you to consider when working up this antibody. However, there is some information that may not be necessary for you to routinely take into account when using the antibody, and you may not have an idea as to what any of this means. Hopefully this article will break down the antibody data sheet and make it all understandable!!

Unfortunately, all manufacturers' primary antibody data sheets are not equal. There are no regulations in place that dictate what information must be stated on each sheet. We are lucky that many of the prominent immunohistochemistry (IHC) vendors in the clinical anatomic pathology market provide the same pertinent information. But since there are no regulations, the format of these data sheets is not uniform and information is sometimes buried in the document.

- antibody name
- manufacturer's catalog number
- clone
- source
- epitope
- reactivity
- isotype
- immunogen
- intended use
- description
- positive control
- staining pattern
- storage requirements
- staining recommendations

What should at least be stated for each antibody is:

Name- The **name** of the antibody is determined when a researcher obtains a license for making their antibody available commercially. They create the name, which can basically be anything (any length of numbers or letters with dashes and periods, etc.). Each antibody is directed against a specific antigen, or a molecule that has the possibility to cause an immune response. On each antigen are areas called **epitopes**, which are a particular combination of amino acids that the binding site of the antibody will bind to on the antigen. Data sheets might list where the epitopes are located on a specific antigen.

Clone- An antibody **clone** is a name for an antibody that binds with a specific epitope, so one general antibody name could have several different clones. An example of this would be Estrogen Receptor, which has clones 1D5, or 6F11, or SP1 among many others. The various antibody clones will bind to different epitopes on the same antigen.

Source- If the antibody was raised in a mouse or a rabbit (the two most common species), this will be listed on the data sheet. It also will list if the antibody is a monoclonal (against one epitope) or polyclonal (against several epitopes). This is important when choosing an antibody (especially if staining is to be done on animal tissue) or when determining what detection system to use.

Reactivity- The species that the antibody is known to react with should be listed on the data sheet. Most antibodies found in the clinical lab will list human tissue as their reactivity, and may list the other animals that it will not work against. If specific animals are in question, call the vendor and they should be able to give more information than listed on the sheet.

Isotype- The isotype of the antibody states to what type of immunoglobulin the antibody-antigen binding will occur and where. Immunoglobulins (Ig) are a family of proteins that antibodies belong to, with a general 4 polypeptide chain configuration. There are 5 types- IgG, IgM, IgA, IgD, and IgE. IgG and IgM are the most common types seen in immunohistochemical reactions. An example of the isotype listing is "**Isotype: IgG2a, kappa,**" which means that the binding will occur on an IgG antibody, and it will be on the kappa light chain. This is important information for determining a negative control to be used. A negative control to use to test the antibody's non-specific binding is a reagent that is raised in the same animal as the antibody with the same isotype. So if the example listed above was a mouse antibody, a negative control reagent to use would be a mouse IgG2a, control (which is a commercially available reagent).

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Immunogen- The **immunogen** is what was supplied to the host animal to elicit an immune response and obtain this antibody. Examples of an immunogen could be a type of cell, or a cell line, a protein fragment or a virus.

Intended use- If the antibody can be used “**For In Vitro Diagnostic Use**”, it will primarily be listed here. If the antibody is “**For Research Use Only**” it will also be listed here. Also the type tissue that the antibody has been tested on by the manufacturer will be listed, such as if it was tested on formalin fixed, paraffin embedded tissue or on frozen sections.

Description- The manufacturer will define the antibody and might give a short introduction as to why the antibody is used.

Positive control- The manufacturer will list a tissue type that is to be used as a positive control for this antibody. That means that if the suggested tissue is used with the antibody at the recommended dilution using the recommended detection system, positive staining should be seen in the positive control. A positive control should be run with each patient test to ensure that the process is working properly. Manufacturers will generally list a normal tissue type that will yield positive staining along with a diseased or tumor tissue type that usually will be the condition that you are staining for and can be used as a control. An example of this would be for cytokeratin 20: a positive control listed might be normal colon, where the staining will be seen in the epithelium, and additionally another positive control might be colon carcinoma where a diseased tissue staining pattern would be seen. These two tissue types would then be recommended as a starting point when working up this antibody and staining might look different between the two.


Staining pattern- The **staining pattern** of the antibody is where the staining should be seen on the tissue. Examples of this can include nuclear, cytoplasmic, or cell membrane staining patterns.

Storage requirements- Generally antibodies are to be stored in a 2 to 8°C refrigerator, however some need to be stored in a -20°C freezer or some even in a -80°C freezer. These requirements are listed along with notes on stability. Antibodies in liquid format usually have a shorter shelf life than antibodies that have been lyophilized, which are in a freeze-dried powder form and need to be reconstituted. If the vendor offers the primary antibody in this format, stability limits should be defined.

Staining requirements- In the staining requirements section, the manufacturer of the primary antibody should detail an IHC staining protocol recommended for

the user to follow to obtain positive staining with this antibody. These conditions are generally those used when this antibody was tested at the manufacturer’s facility and often times have used other reagents and equipment that the manufacturer also offers commercially. This is important to remember, especially when using a prediluted antibody, since their recommended dilution (which is provided to you as a ready-to-use reagent so there is nothing you can alter about the concentration) and incubation time have been optimized using their detection system. The **staining requirements** section should include any pretreatment recommendations, antibody dilution range and recommended diluent, incubation time, detection system, and chromogen. There also may be fixation recommendations, as some antibodies work better when fixed with one fixative over another. Remember that these conditions are recommendations, and are a place for testing to begin in your lab. Any modifications that are made to this recommended protocol, yet still yield optimal results, are acceptable, and the vendors of these products encourage you to make changes as needed to yield good IHC staining.

There is one exception to changing recommended protocols provided on a primary antibody data sheet- this is when using FDA approved kits. Examples of such kits are Dako’s HercepTest™, which aids in finding patients with breast cancer that will benefit from Herceptin treatment, or Dako’s EGFR kit which aids in finding patients with colorectal cancer that are eligible for Erbitux treatment. These kits are complete systems that have been FDA approved, which basically means that stringent testing was done to prove to the FDA that the method, yielding a specific outcome, is valid. When testing is complete, a very strict procedure must be followed and dictated to customers for individual lab use. Examples of conditions that must be complied with are type of fixative, length of fixation, method of epitope retrieval, retrieval solution used, and step-by-step incubation times, among others. When one of these kits is being used, no variability to the procedure is tolerated; these procedures are to aid in defining treatment for the patient and must strictly be followed.

The primary antibody data sheet is an important piece of literature for your lab. A wealth of information is held here (many vendors offer more information than listed above) and is meant to help you to optimize the antibody in your lab with your staining conditions. Start with these recommendations and use a routine antibody work-up protocol that is specific to your lab along with any special requirements that your lab may have. Don’t be afraid to make modifications to the protocols recommended by the vendor, as the data sheet is a starting place to optimization of your new antibody. 



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- 1. Since one antigen has several different epitopes that antibodies can bind to, each antibody to that antigen can have several different:
A. Clones
B. Controls
C. Immunogens
D. Sources
- 2. IHC tests that MUST be performed EXACTLY as dictated by the antibody manufacturer are those that are:
A. FDA Approved
B. For In Vitro Diagnostic Only
C. For Research Use Only
D. Prediluted Antibody
- 3. TRUE or FALSE (circle one): Only normal tissue should be used as positive controls.
- 4. When looking to find out if an antibody will bind non-specifically to the animal tissue section, look on the antibody technical data sheet for information about:
A. Immunogen
B. Isotype
C. Name
D. Reactivity

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