

So you want to do an immunohistochemistry assay?

If the antibody has not been tested on tissues before, it will take quite a bit of effort to determine if the antibody is specific--- the assays will need to be optimized.

There should be a plan

- for negative and positive control reagents,
 - tissue negative and positive controls,
 - secondary reagents,
 - blocking reagents
 - are overnight incubations needed?
 - will a fluorescence OR enzyme detection system be used?
 - will amplifications such as antigen retrieval
 - or will catalyzed signal amplification be needed?
- etc. etc.

You may find some hints on the powerpoint presentations on the histology core website:
<http://cancer.ucsd.edu/histology/>

Other Questions:

1. Are you going to immunostain frozen sections or paraffin sections?
2. Is your antibody a polyclonal rabbit or polyclonal goat or mouse monoclonal or rat monoclonal?
3. What is the tissue you are testing, rabbit tissue or rat tissue or mouse tissue?
4. Is the antibody sold as being tested and shown to work well in immunohistochemistry assays?
5. What is the expected Positive control tissue for the antibody?
6. What is the expected Negative control tissue for the antibody?
7. Do you have cells in tissue culture that you know are positive or negative for the antibody that can be used as controls in the assay?
8. What is the concentration of antibody that you used on Westerns to obtain the positive staining?