

IHC checklist

Slides/antibodies must be in hand before the IHC request is entered into iLab. Please wait for a reply from us as to what date will work for us and you. We can only run 36 slides at a time and may be booking several people on one run.

Slides and pre-diluted antibody are submitted the afternoon/evening before the scheduled run.

Antibody is diluted with TBST (Tris buffered saline w/ Tween 20) and submitted in a vial provided by KI Histology core. (the vials are specific for our IHC stainer). The vial must be placed into our refrigerator and be labeled with antibody and initials of researcher. Slides can go on the counter above the refrigerator.

HIER citrate buffer, secondaries and chromogen are provide by the Core, unless you want to use your own secondary, in which case the charge per slide will be reduced.

Volume needed is 300 ul per slide, plus a bit extra. (at least 600ul extra per vial. The IHC stainer does not like "close" volumes.)

We carry HRP mouse, rabbit, rat and goat. We also carry AP for mouse and rabbit. The only 2 primaries we carry are cleaved caspase 3 and Ki-67. If you require a different secondary or fluorophore, you will need to provide it. (we will reduce the cost per slide if you provide these

If you are submitting a large volume of rat or goat primary antibody slides for staining, please notify us several days before hand, as we do not keep larger volumes of the secondary reagents on hand, as they are not highly used.

Please drop by and chat with us about fluorescent staining and double staining. These require strategic planning.

All formalin fixed paraffin embedded slides are antigen retrieved using heat induced epitope retrieval at 97° C for 20 minutes using citrate buffer ph 6. If any other ph is required, the solution must be provided to Histology.

The Histology Core standard automated IHC (ThermoScientific IHC Autostainer 360) run consists of:

Endogenous peroxidase blocking: 10 minutes
Protein block: 30 minutes
Primary antibody: 60 minutes
Labeled polymer: 15-30minutes

DAB: 5 minutes

Other programs are available for other species/primaries, please contact the Histology lab to discuss options.

We strongly recommend that you run a known positive and a known negative control with every run.

We will charge you even if your run doesn't work, we suggest you run a couple of test slides before you ask for a full run of 36 slides.

You are always welcome to book time w/ Dr. Bronson our veterinary pathologist to confirm your IHC staining results.

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