

Translational Pathology Imaging Laboratory (TPIL)

Manager: Marion Joy, PhD

joym@upmc.edu

| Principal Investigator Information | | | | |
|--|--|------------------------------------|--|--|
| PI Name: | Email: | | | |
| Contact Person: | Email: | | | |
| Billing Account Number: | | | | |
| billing Account Humber. | | | | |
| Research Synopsis | | | | |
| Example: Interrogation of immune infiltrate of | breast carcinoma and surrounding st | roma based on estrogen receptor | | |
| status | | | | |
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| Study Details | | | | |
| Species: | Tissue Type: | Tissue Type: | | |
| Number of Slides: | Tissue Microarray | Tissue Microarray Specimen: Yes No | | |
| Antibody Targets: Up to 6 per panel; DAPI included on all panels | | | | |
| Panel 1 | | | | |
| Panel 2 | | | | |
| Panel 3 | | | | |
| Target Area: Tumor Only Normal | Only Tumor vs stroma Tumo | or vs distant normal | | |
| Number of ROIs per specimen: | | | | |
| Pathology Review Needed: Yes | No Bioinformatics Su | pport Needed: Yes No | | |
| Tathology neview needed. | Separate fee; NOT included in estimate | | | |
| Timeline for Project Completion: | | | | |
| Date to target for grant submission, manuscript completion, etc. | | | | |
| FEDE Slides | | | | |
| | | | | |

FFPE Slides

Freshly Cut Unstained Slides Needed: Yes No

Separate fee; NOT included in estimate. See FAQ #8 for details.

Number of Subjects: Unstained Slides Per Subject:

H&Es Needed: Yes No



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FAQ

1. Our group has an antibody that we would like to include in the panel. Is that possible?

Yes, as long as the antibody is derived from a rabbit or mouse, TPIL will attempt to optimize the antibody and panel and review results optimization results prior to proceeding with the project. Note, there is a billing charge for optimization. There will be additional charges if pathology review is needed.

2. What antibodies have been optimized in TPIL? Refer to page 3.

3. What is required to optimize a new panel/antibody?

For new antibody panels/ addition of antibodies not previously worked with in the laboratory, we will need to optimize the panel/antibody. For antibodies with well-established targets (e.g. SOX10), this usually requires three (3) to five (5) blank FFPE slides that represent the tissue to be targeted (e.g. chondrosarcoma tissue if working with chondrosarcoma tissue microarray [TMA]) cut at 4-5µm thickness. Note, that for carcinoma cases we normally recommend pancytokeratin (panCK) to be on the panel especially for projects wanting to delineate stroma versus tumor microenvironment. Additionally, if an antibody is being optimized for which targets are not well-established or utilized in a tissue in which antigen levels are not well-described, TPIL would ideally want validated positive and negative controls or wild-type and knockout tissues for optimization.

4. After optimization, what does TPIL need to proceed with the project?

We will need two blank FFPE slides with 4-5µm thick tissues per sample (one slide is for staining the other is a backup in case of issues with the initial staining). We prefer all slides (for optimization and project) to be delivered together to prevent delay between optimization and project staining. All slides and additional materials should be delivered to Marion Joy, PhD Shadyside Hospital Room WG23. Drop off hours are Mon, Tues, Fri from 8am-1pm.

5. Can I approve/select regions of interest for analysis?

Yes, TPIL is able to set up additional meetings to ensure that regions of interest are targeted appropriately. Note, this may result in additional costs for the project.

6. What do I get at the end of analysis?

An excel worksheet with data regarding regions of interest as well as images will be provided. TPIL can usually upload these files via OneDrive, but larger studies will require the researcher to provide TPIL with an external drive.

7. How do I know if I need bioinformatic analysis?

Basic quantification of positive cells staining with markers are easily done. However, more complex analyses (e.g. comparing nearest neighbors for tumor cells or multiple regions of interest or TMA tissues) usually is aided by bioinformatic support.

8. I don't have my own unstained slides and need to request them. How do I do this? What are the fees? The UPMC Clinical Test Development Lab is able to cut slides for your project. Please complete the "FFPE Slides" portion of the request form, and send it to Kate Smith at smithkm13@upmc.edu. A quote will be provided upon receipt.



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| Optimized Antibodies (Human) | | | | |
|------------------------------|--------|-------|-------|--|
| CD3 | CD20 | OX40 | IL21R | |
| CD8 | CD4 | LAG3 | B7-H3 | |
| PD-L1 | MHCII | TIM3 | Ki67 | |
| PD-1 | CD16 | HER2 | PNAd | |
| CD68 | CD45RO | CD31 | AID | |
| CD163 | CD11c | CD47 | SOX10 | |
| CD206 | CD56 | CD160 | panCK | |
| FOXP3 | CTLA4 | T-bet | | |
| Optimized Antibodies (Mouse) | | | | |
| CD3 | | CD11b | | |
| CD8 | | CD11c | | |
| CD161 | | Ly6G | | |
| panCK | | BCL6 | | |
| FOXP3 | | CD19 | | |
| F4/80 | | LYVE1 | | |
| | CD4 | | | |