Spatial Tissue Exploration Program (STEP) Project Requirements & Tissue Preparation Details.

STEP for PhenoCycler® Open (coverslips)

1. Once a purchase order has been submitted/processed, Akoya Biosciences will send poly-l-lysine coated coverslips in a coverslip storage box.
2. Customer will send 3x the number of coverslips per assay. Coverslips should not be etched or marked in any way. The coverslip storage box ***MUST*** be clearly labeled with the appropriate project number (begins with AK). A tissue map with all corresponding sample IDs ***MUST*** be included in the coverslip storage box before shipment to Akoya Biosciences.
3. Customer will section tissue samples onto the coverslips and ship them to Akoya Biosciences:

   ATTN: Sejal Mistry
   Smistry@akoyabio.com
   1080 O'Brien Dr. Suite A
   Menlo Park, CA 94025.

   Note the following tissue preparation details and considerations:
   a) A maximum of 2 samples/coverslip will be accepted to ensure success with the *PhenoCycler® Open workflow*.
   b) Tissues must fit within an approximate 15x15 area in the middle of the coverslip. Any tissue which falls outside of this imageable area will not be able to be imaged or analyzed.
   c) The customer shall refer to tissue processing best practices provided by Akoya Biosciences. Page 29-30 of the PhenoCycler® manual explains sectioning and coverslip preparation. If appropriate sample preparation techniques and pre-analytical considerations are not adopted by the customer, it is possible for a tissue to detach from the coverslip. Factors influencing section detachment include but are not limited to section thickness (Akoya Biosciences recommends section thickness of ~5-10um), human influence, and size and fixation of the samples. If section detachment occurs before the PhenoCycler® assay is carried out, Akoya Biosciences will request that the customer sends new coverslips. If section detachment occurs during the PhenoCycler® assay and samples are lost, Akoya Biosciences will not re-run the assay, and provide results on all remaining tissues.
   d) All frozen samples MUST be shipped to Akoya Biosciences on dry ice to ensure the integrity of the samples for the PhenoCycler® assay.

STEP for PhenoCycler® Fusion (slides)

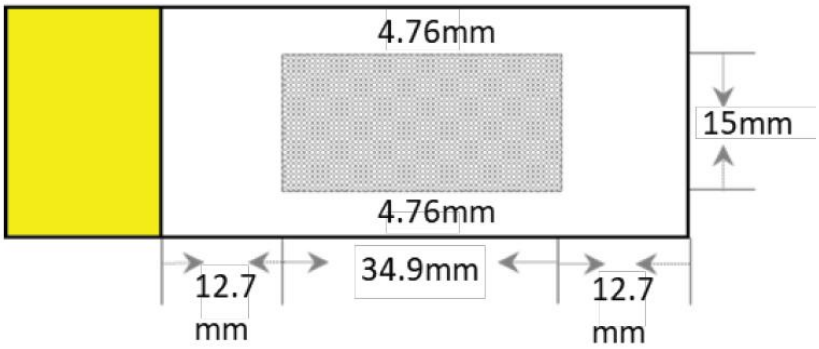
1. Customer will send 3x the number of slides per assay. The slide storage box ***MUST*** be clearly labeled with the appropriate project number (begins with AK). A tissue map with all corresponding sample IDs ***MUST*** be included in the slide box before shipment to Akoya Biosciences.
2. Customer will section tissue samples onto the slides and ship them to Akoya Biosciences:

   ATTN: Sejal Mistry
   Smistry@akoyabio.com
   1080 O'Brien Dr. Suite A
   Menlo Park, CA 94025.

Note the following tissue preparation details and considerations:

a. For PhenoCycler®-Fusion experiments, fresh-frozen or FFPE tissues are sectioned and directly adhered to standard sized (1" x 3") glass super frost charged microscope slides. Using non-standard microscope slides and/or tissue preparation techniques that deviate from this protocol are not compatible with the PhenoCycler®-Fusion. Please refer to ***Chapter 2 of the PhenoCycler® Fusion User Guide for Sample Preparation Guidelines for FF (page 31-33) and FFPE samples (page 34-36).*** Each slide should be labeled with the appropriate sample ID. If more than 1 sample is placed on a slide, each slide should be labeled with all the sample IDs on that slide in a way that is clearly identifiable. Alternatively, the inside of a slide box can be labeled with all Sample IDs corresponding to each slot.

b. Tissues must fit within an approximate 15x34.9mm area in the middle of the slide (refer to image below). Any tissue which falls outside of this imageable area will not be able to be imaged or analyzed.



c. If appropriate sample preparation techniques and pre-analytical considerations are not adopted by the customer, it is possible for a tissue to detach from the slide. Factors influencing section detachment include but are not limited to section thickness (Akoya Biosciences recommends section thickness of ~5-10um), human influence, and size and fixation of the samples. If section detachment occurs before the PhenoCycler® assay is carried out, Akoya Biosciences will request that the customer sends new slides. If section detachment occurs during the PhenoCycler® assay and samples are lost, Akoya Biosciences will not re-run the assay, and provide results on all remaining tissues. To ensure tissue adherence for problematic tissue types, Akoya Biosciences recommends coating the slide in poly-L-lysine before sample preparation.

d. All frozen samples MUST be shipped to Akoya Biosciences on dry ice to ensure the integrity of the samples for the PhenoCycler® assay.

STEP for Custom Conjugation (STP2000)

1. The customer will ship purified materials to Akoya Biosciences at the following address:
   ATTN: Sejal Mistry
   Smistry@akoyabio.com
   1080 O'Brien Dr. Suite A
   Menlo Park, CA 94025.
2. Requirement and Recommendations:
   a. A minimum of 50ug of purified material is required for custom conjugation. This

will yield ~20+ reactions per conjugated antibody depending on the antibody, as well as its yield and titer (based on starting dilution of 1:50 for each barcoded antibody). The customer should further optimize this dilution for optimal performance. The customer can request to custom conjugate 100ug of purified material, which will yield ~40+ reactions per conjugated antibody depending on the variables outlined above.

b. Purified materials must be free of carrier proteins (primarily BSA) and other chemicals and stored in PBS or in a similar buffer for optimal performance. Lyophilized antibodies should be avoided, as they generally result in poor conjugation efficiency. ***Please refer to the guidelines in the PhenoCycler® Fusion User Guide, page 96.*** If purified clones are not commercially available, the customer may request that the antibody vendor completed a purification process. Alternatively, the customer can complete this purification process using commercially available purification kits. Once any kind of purification is performed, the concentration of the purified antibody must be measured again. Please note that concentrations listed on commercially available antibodies are usually not accurate and could result in poor conjugation efficiency.

3. Protein gel electrophoresis will be performed for all custom antibodies to verify the success of the antibody conjugation reaction. Akoya Biosciences will not perform antibody validation or antibody titration as part of the custom antibody conjugation service. Akoya Biosciences can provide a PhenoCycler® antibody validation technology note and recommendations upon request. ***Refer to Appendix D: Validating custom conjugated antibodies, page 21 of the PhenoCycler® Fusion User Guide.*** All antibodies should be titrated by the customer upon receipt of the conjugated antibodies. ***Refer to Appendix C: Titration of PhenoCycler® antibodies, page 120 of the PhenoCycler® Fusion User Guide.*** Protein gel electrophoresis results only assesses the success of the chemical reaction used for barcode-antibody conjugation. Overall success of custom conjugated should be further evaluated via antibody staining in tissue samples. Antibody characterization and performance QC can be performed on the customers' tissues using STEP+ Conjugated Antibody Testing (STP2001). Due to variability in antibody formats and performance, no guarantees can be made regarding success of conjugation and inclusion of conjugated antibody in the PhenoCycler® run. Akoya will test a maximum of two clones per custom antibody; both clones will be provided by the customer. Each custom conjugation has an approximate efficiency of 60-70%.
4. Upon completion of conjugation and optional *in situ* screening of antibodies, the customer will obtain conjugated antibodies (barcoded antibody with a corresponding reporter) for use in their laboratory, as well as a custom antibody conjugation report. This report will provide an analysis on the efficiency of the chemical conjugation.
5. Each custom conjugated antibody has an approximate shelf-life of at least 6 months at 4˚C.