**Protocol CyTOF004.4: Metal Labeling 200μg IgG CyTOF Antibodies with Cisplatin**

Indication: for preparing cisplatin-conjugated antibodies for CyTOF analysis (200μg)

Note that this protocol requires “carrier-free” IgG for labeling (meaning no BSA, hydrolyzed protein, gelatin, etc. for stabilization). Most IgGs stored in the presence of carrier contain BSA. Many companies offer custom preparation of carrier-free versions of antibodies normally containing BSA. While expensive, this option is very useful when no other alternatives are available.

**Note:** This protocol is optimized for a multitude of IgG isotypes and also works well for affinity purified polyclonal preparations – **will not work for IgM mAbs**

**Reagents and Materials:**

* 200μg **carrier-free** IgG (MW ~180,000; 1.1x10-9 moles)
* 0.5M Neutral TCEP (Tris-carboxyethyl phosphine): 10μL aliquots
* Buffers:
  + R-Buffer
  + C-Buffer
  + L-Buffer
  + W-Buffer
* Purified cisplatin isotope solutions (1mM stocks – Standard BioTools) – four available: 194Pt, 195Pt, 196Pt, 198Pt. This protocol can also be used for antibodies coupled to natural abundance cisplatin (isotope mixture).
* TBS or PBS-based antibody stabilization solution (Candor) – *Note: this can be supplemented with 0.2% azide after purchase.*
* 50kDa MWCO Amicon Ultra 500μL V-bottom centrifugation columns (3 per labeling reaction)
* Screw top Eppendorf tubes
* Filter Tips (to prevent cross-contamination between reagents)

**Before Starting:** Equilibrate all conjugation buffers to RT

**Protocol and Timing: 4 – 4.5 hours total**

**0:00 *Buffer exchanging the antibody.***In a 50kDa MWCO micro-filter device, add 300μL of R-buffer then 200μg of antibody (Max Volume 500μL). If 200μg of antibody accounts for greater than 200μL of volume, either pre-concentrate it in the same 50kDa MWCO column, or reduce the volume of R-buffer accordingly. Centrifuge at 12,000xg at RT for 8 min. Discard flow-through (ideally the final volume should be 20μL or less).

**0:15 *Partial antibody reduction.***Mix 8μL of TCEP stock with 992μL of R-buffer (4mM final concentration). Remove the concentrated antibody (~20µL) from the bottom of the 50kDa MWCO tube and transfer to a new 1.5mL microcentrifuge tube. Rinse the sides of the filter in the 50kDa MWCO tube with 100μL of the diluted TCEP 5 times, then transfer to the antibody in the 1.5mL microcentrifuge tube. Pipette up and down 5 times to mix. Incubate microcentrifuge in a 37°C water bath for 30 min.

**1:00 *Clean up partially reduced antibody.*** Collect the partially reduced antibodies from the 37°C water bath. Add 300μL of C-buffer to the partially reduced antibody and mix by pipetting up and down 5 times. Transfer mixture to a new 50kDa MWCO tube. Reduce the volume by centrifugation at 12,000xg for 8 min at RT. Discard flow-through and repeat wash and spin with an additional 400μL of C-buffer.

**1:30 *Coupling antibody and cisplatin.*** Remove the partially reduced antibody (~20µL) from the bottom of the 50kDa MWCO tube and transfer to a microcentrifuge tube. Rinse the sides of the filter in the 50kDa MWCO tube 5 times with 100μL C-buffer and transfer to the microcentrifuge tube. In a separate microcentrifuge tube, dispense 40µL 1mM cisplatin isotope solution (20µL aliquots at -20°C), and dilute with 840µL C-buffer. Add entire diluted cisplatin solution (880µL) to the partially reduced antibody and pipette up and down to mix (total volume ~1mL). Incubate at 37°C for 90 minutes.

**3:00** ***Washing cisplatin-conjugated antibody.*** Transfer 500µL of the cisplatin-conjugated antibody solution to a new 50kDa MWCO tube. Centrifuge at 12,000xg for 8 min. Discard flow-through. Transfer remaining 500µL of cisplatin-conjugated antibody solution to the same 50kDa MWCO tube and centrifuge again. Discard the flow-through. Wash 3 times with 400μL of W-buffer.

**4:00 *Recovery of cisplatin-conjugated antibody.***Remove the concentrated labeled antibody (~20µL) from the bottom of the 50kDa MWCO tube and transfer to a new 1.5mL microcentrifuge tube. Wash the sides of the filter in the 50kDa MWCO tube 5 times with 100µL W-buffer and add to the microcentrifuge tube. Pipette up and down 5 times to mix.

**4:10 *Quantification.***Quantify antibody using the Qubit 4 Fluorometer. Prepare sufficient 1X protein assay reagent for at least 210μL per conjugated antibody (1:200 dilution of 200X protein assay reagent in assay buffer). Transfer 1μL conjugated antibody solution to the bottom of a Qubit assay tube. Add 199μL 1X protein assay reagent and pipette up and down to mix. Incubate 15 minutes at room temperature and measure on the Qubit 4 using the “Protein” function, indicating 1μL volume used. An expected recovery should be about 60% (60μg). Calculate recovery assuming 120μL final volume. Add sufficient antibody stabilizer to bring the concentration down to 0.2mg/mL. Store at 4°C.