Standard Operating Procedure:

Antigen Purification

Steps to do before the selection:

1. Preparation of Solutions

IMAC lysis buffer: 50mM Tris-HCl, 300 mM NaCl, 5% glycerol, 10 mM imidazole, 0.5 mM TCEP, 0.05% Triton X100 **pH 8.0**

IMAC wash 1 buffer: 50mM Tris-HCl, 300 mM NaCl, 5% glycerol, 10 mM imidazole, 0.5 mM TCEP, **pH 8.0**

IMAC wash 2 buffer: 50mM Tris-HCl, 300 mM NaCl, 5% glycerol, 20 mM imidazole, 0.5 mM TCEP, **pH 8.0**

IMAC elution buffer: 50mM Tris-HCl, 300 mM NaCl, 5% glycerol, **300 mM imidazole**, 0.5 mM TCEP, pH 7.5

Gel filtration buffer: 20 mM HEPES, 300 mM NaCl, 5% glycerol, 0.5 mM TCEP, pH 7.5

2. Samples preparation

1). The frozen cell pellets are briefly thawed in warm water. Resuspend the cell pellets by adding Lysis buffer to final volume 100 mL/(per liter culture), Freshly add PMSF and Benzamidin to a working concentration of 1mM and add Benzonase to 5U/ml.

2). Sonication with program nr 1 (5s/7s, 10 min, ~100W) for lysate from 2 L cultivation.

3). The sonicated lysates are centrifuged for 60 min at 16000 rpm in the JLA 16.250 rotor. The soluble fraction is decanted and filtered through 0.45μm filters.

3. Purification

Purification is performed using a two-step process, IMAC + Gel filtration, on the ÄKTA Xpress system (GE Healthcare). The 5 mL chelating columns and gel filtration columns were equilibrated with IMAC wash 1 buffer and Gel filtration buffer, respectively.

Purification programs:

Chelating 5ml GFS75 2nd wash 20CV level and slope

Chelating 5ml GFS200 2nd wash 20CV level and slope

1. Filtered samples are loaded to the IMAC columns on position 1-4 from sample inlets S1-S4
2. IMAC columns are washed by 10 CV of IMAC wash 1 buffer and 10 CV of IMAC wash 2 buffer.
3. IMAC columns are eluted by 5 CV IMAC elution buffer, and major peak is loaded to gel filtration columns on position 5.
4. The fractions from gel filtration column are checked by SDS-PAGE and pool the pure fractions together. The antigens are further validated by Mass spectrum.
5. The pure antigen samples are diluted or concentrated to 1 mg/ml and make 100 uL aliquots. The aliquots are flash freezed by liquid nitrogen and ship to binders lab with dry ice.

Fab Purification

Steps to do before the selection:

1. Preparation of Solutions

Protein A lysis/wash buffer: 1x PBS, 0.05% Triton X100, 5% glycerol, pH 7.4.

Protein A Elution buffer: 100 mM Acetic acid, pH ~ 2.5

SP buffer A: 50mM NaAc/HAc, 5% glycerol, pH 5.0

SP buffer B: 50mM NaAc/HAc, 5% glycerol, 1M NaCl, **pH 5.0**

2. Samples preparation

1. The frozen cell pellets are briefly thawed in warm water. Resuspend the cell pellets by adding Lysis buffer to final volume 50 mL/(per liter culture), Freshly add PMSF and Benzamidin to a working concentration of 1mM and add Benzonase to 5U/ml.
2. Sonication, total 8 min, 4sec on 5sec off, ~100W
3. The sonicated lysates are centrifuged for 30 min at 49000 xg rcf. The soluble fraction was decanted and filtered through 0.45μm filters.
4. The Protein A and SP columns were already equilibrated with buffer.

3. Purification

Purification is performed using a two-step process, Protein A affinity chromatography + ion exchange, on the ÄKTA Xpress system (GE Healthcare). The 1 mL Protein A columns and SP columns were equilibrated with Protein A lysis buffer and SP buffer A, respectively.

Purification programs:

ProteinA 1ml SP 2nd wash 20CV level and slope

1. Filtered samples are loaded to the Protein A columns on position 1-3 from sample inlets S1-S4
2. Protein A columns are washed by 10 CV of IMAC wash 1 buffer and 10 CV of protein

A wash buffer.

3. IMAC columns are eluted by 5 CV protein A elution buffer, and major peak is loaded to SP column on position 4.

4. The fractions from SP column are checked by SDS-PAGE and pool the pure fractions together. The Fabs are further validated by Mass spectrum.

5. The pure Fabs are diluted or concentrated to 0.5 mg/ml and send to secondary validation lab.

IgG Purification

Steps to do before the selection:

1. Preparation of Solutions

Binding and washing Buffer: 1x PBS, pH~7.4

Elution Buffer: 100mM Acitic acid, pH 2.5

Neutralization buffer: 1 M Tris-HCl, pH 9.0

Dialysis buffer: 1x PBS pH~7.4, 0.09% sodium azide

2. Purification Step

1. Transfer medium with secreted IgG to 200 mL centrifuge tubes and centrifuge them at 38000 xg rcf 0.5h.
2. Transfer supernatant to centrifuge-cones and add 1/10 volume 10x PBS and 2mL protein A slurry per 200 mL medium.
3. Do batch binding in cold room 1h
4. Centrifuge centrifuge-cones at 2000 rpm, 5 mins, and decant supernatant. Transfer the protein A beads to open columns.
5. Wash Protein A beads by adding 10-folds volume washing buffer.
6. Elute Protein A beads by 1 mL*5 Elution buffer and neutralize it by 1/10 vloume of neutralization buffer
7. Dialyze elutions in 6L pre-chilled dialysis buffer O.N. in coldroom.
8. The IgGs are further validated by Mass spectrum.
9. The pure IgGs are diluted or concentrated to 0.5 mg/ml and flash freeze by liquid nitrogen.