

Pullouts with IgG Resin

Necessary reagents and instrumentation:

Extraction buffer(s):

1. 20mM Hepes pH7.4, 110mM KOAC, 150mMNaCl, 1% Triton, 1mM DTT, 1:100 SolutionP, 1:200 PIC
2. 20mM Hepes pH7.4, 0.1mMMgCl₂, 0.5%NaDoc, 0.01% SLS. 1mMDTT, 1:100 Solution P, 1:200PIC

Wash buffer(s):

1. 20mM K/Hepes pH7.4, 0.1mM MgCl₂, 0.1% Tween 20, 1mM DTT, 1:100 Solution P
2. 0.1M NH₄OAc/0.1mM MgCl₂

Elution buffer(s)

1. 0.5M NH₄OH/0.5mM EDTA
2. 0.5 M HAC

Protocol

Start with 2.5 grams of frozen cell powder. Weigh out in a 50ml falcon tube. Thaw. Add 25ml of extraction buffer @RT to the pellet. Vortex

For Example **extraction buffer** for **Nic96**: 20mM Hepes pH7.4, 110mM KOAC, 150mMNaCl, 1% Triton, 1mM DTT, 1:100 SolutionP, 1:200 PIC

For Example **extraction buffer** for **POM34**: 20mM Hepes pH7.4, 0.1mMMgCl₂, 0.5%NaDoc, 0.01% SLS. 1mMDTT, 1:100 Solution P, 1:200PIC

Let cell powder thaw. Resuspend by vortexing

Polytron at settings 5.0 for 30 seconds. Keep samples on ice

Place tubes on rotating wheel. Incubate for 1 hour at 4°C

Centrifuge 3000 rpm for 15 minutes

Transfer supernatant to Beckman Ty50.2 tubes and centrifuge 48,000 for 1 hour at 4°C

Transfer supernatant to fresh falcon tubes. Add IgG sepharose 50ul slurry for 2.5 grams of cell powder

Incubate rotating at 4°C overnight

Next day pellet resin by spinning at 3000rpm for 1 minute

Aspirate off supernatant

Transfer the resin to the siliconized tubes (Fisher 02-681-331)

Wash 3 times with 1 ml **Wash Buffer**

Wash Buffer: 20mM K/Hepes pH7.4, 0.1mM MgCl₂, 0.1% Tween 20, 1mM DTT,
1:100 Solution P

Transfer the resin to a fresh siliconized tube and wash 3 more times with a wash buffer

Transfer the resin to a spin column. Wash the column with wash buffer

Wash the resin 150ul x 3 with 0.1M NH₄OAc/0.1mM MgCl₂

Elute with 0.5M NH₄OH/0.5mM EDTA

To make Buffer:

338ul NH₄OH (concentrated 14.8N)

10 ul 0.5MEDTA

9.652 ddH₂O

Check pH-10.9

Or Elute with 0.5 M HAC

To make 100ml mix 2.87 ml 100% HAC with 75 mls distilled water and bring pH to 3.4 with ammonium acetate solution (0.5M), takes 6-7 ml, then vol tot 100 mls.

NH₄OAc- to make 50ml of a 4M solution. Dissolve 15.41 g NH₄OAc in ddH₂O and should be adjusted to 7.4-

Freeze elution in liquid nitrogen

Speedvac for 4 hours or overnight