

- Ni-NTA resin is stored at 4 degrees as 50% slurry in 20% ethanol
  - Centrifuge for 2 min. at 700 xg
  - Remove supernatant
  - Add 2x resin-bed volume of His Wash buffer 2 centrifuge for 2 min at 700 xg, remove supernatant and repeat
  - Resuspend resin with the protein lysis supernatant
  - Stir 100 ml lysis solution with 7 ml Ni-resin (not slurry) in a 250 ml glass beaker in the cold room for 30 min, under gently magnetic stirring (= batch binding)
  - Wet the filter in the yellow glass column with tap water
  - Pour supernatant and resin to the column, collect the flow through
  - Take 10 ul flow through for SDS-PAGE
  - Wash with 20 column volumes (150 ml) of wash buffer 1 and 10 column volumes (75 ml) of wash buffer 2.
  - Use Bradford to check if wash buffers don't contain protein anymore
  - Take out 10 ul wash buffer and 10 ul resin for SDS-PAGE
  - Add elution buffer (contains 250 mM imidazole)
  - Save elution steps in eppendorf tubes (25x ~1.5 ml). Protein elutes in 40 ml
  - Take 10 ul of elution for SDS-PAGE
  - Pool steps that contain protein (~2 - 18)
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- Resin can be used at least 5x before regeneration
  - Wash resin with 10x C.V. MES 20 mM pH 5, 100 mM NaCl and 10x C.V MQ water.
  - Store as 50% slurry in 20% ethanol in cold room

#### For uncleaved Ras

- Buffer exchange to reduce imidazole amount
- Use Millipore 10K centrifugal filter 4000 xg for 15 min. to concentrate (to ~ 5ml). Add wash buffer 2 and mix! Repeat 1x. Concentrate to < 10 ml (= max for gel filtration chromatography)
- Sample to Akta (S75 column)

#### To cleave His-tag Ras

- Concentrate to ~10-15 ml
- Add 50 uM GDP & 10 ul thrombin (scales with cell culture volume)
- Dialyse overnight in 4L general dialysis buffer
- Take out 20 ul for SDS-PAGE sample
- Transfer to 50 ml falcon
- Add 15 ul thrombin, leave at RT for 3h (gently invert every 30-60 min)
- Put protein on ice
- Run SDS-PAGE to confirm cleaving of the His-tag, otherwise leave at RT for another hour
- Concentrate to < 10 ml
- Sample to Akta (S75 column)

- His Wash buffer 1Liter

|                          |  |
|--------------------------|--|
| Tris pH 8, 50 mM         | 50 ml from 1 M stock                   |
| NaCl, 500 mM             | 166.7 ml from 3M stock                 |
| Glycerol, 10%            | 200 ml of 50% stock                    |
| Imidazole, 10 mM         | 10 ml from 1 M stock pH 8              |
| MgCl <sub>2</sub> , 5 mM | 5 mL from 1 M stock                    |
| Add b-ME 10 mM           | x mL from 14.3 M stock just before use |

- His wash buffer 0.5 Liter

|                          |                                      |
|--------------------------|--------------------------------------|
| Tris pH 8, 50 mM         | 25 ml from 1 M stock                 |
| NaCl, 150 mM             | 25 ml from 3 M stock                 |
| Glycerol, 10%            | 100 ml from 50% stock                |
| Imidazole, 10 mM         | 5 ml from 1 M stock                  |
| MgCl <sub>2</sub> , 5 mM | 2.5 ml from 1 M stock                |
| Add b-ME, 10 mM          | x mL from 14.3 stock just before use |

- His Elution buffer 0.5 Liter

|                          |  |
|--------------------------|--|
| Tris pH 8, 50 mM         | 25 ml from 1 M stock                   |
| NaCl, 150 mM             | 25 ml from 3 M stock                   |
| Glycerol, 10%            | 100 ml from 50% stock                  |
| Imidazole, 250 mM        | 125 ml from 1 M stock                  |
| MgCl <sub>2</sub> , 5 mM | 2.5 ml from 1 M stock                  |
| Add b-ME 10 mM           | x mL from 14.3 M stock just before use |

- Kras gel filtration buffer 1 liter

|                           |                           |
|---------------------------|---------------------------|
| HEPES, pH 7.4, 20 mM      | 20 ml from 1 M stock      |
| NaCl, 100 mM              | 33.33 mL from 3 M stock   |
| MgCl <sub>2</sub> , 5 mM  | 5 ml from 1 M stock       |
| TCEP 2 mM                 | 0.5733 gram (Mw = 286.65) |
| TCEP reduces the pH       |                           |
| Add up to 750 mL MQ water |                           |
| Ajust pH to 7.4           |                           |
| Fill to 1L                |                           |

- General dialysis buffer 1 liter

|                          |  |
|--------------------------|--|
| Tris pH 8, 50 mM         | 50 ml from 1M stock                                    |
| NaCl, 150 mM             | 50 ml from 3 M stock                                   |
| Glycerol, 2%             | 40 ml from 50% stock                                   |
| MgCl <sub>2</sub> , 5 mM | 5 ml from 1 M stock                                    |
| DTT, 1 mM                | 1 ml from 1M stock (add just before starting dialysis) |