**Protocol for purification of recombinant protein from 300 mL yeast culture**

**Equipment and reagents needed:**

Zirconia beads (0.5 mm diameter from BSP, Germany)
Paint Shaker (at 4°C)
Tube rotator for 15 mL and 50 mL tubes
Ice bucket
Ice
Disposable pipettes (25 mL and 10 mL)
50 mL tubes
15 mL tubes
Automatic Pipettor
Centrifuge
10,000 MWCO PES concentrator with 4mL capacity
Micro tubes
Vacuum
Permanent Marker or Label System
Yeast clone harvested pellets (fresh or stored at -80°C)
Glutathione Sepharose-4B beads (GE Healthcare, USA)
Lysis buffer without and with protease inhibitors

| Reagent | Amount |
|---|---|
| Tris, pH 7.5 | 50 mM |
| NaCl | 100 mM |
| EGTA | 1 mM |
| Glycerol | 10% |
| TritonX-100 | 0.1% |

Use this recipe to prepare the lysis buffer without protease inhibitors. Add 2 tablets of Roche protease inhibitors, 50 µL of Beta-mercaptoethanol (BME), and 0.5 mL of PMSF (100 mM stock) for every 50 mL of lysis buffer to prepare the lysis buffer with protease inhibitors. Dissolve the tablets completely and mix well.
Roche protease inhibitor tablets (EDTA free)
100 mM PMSF in isopropanol
200 mM Glutathione
500 mL deionized $H_2O$ stored at 4°C
Wash Buffer I

| Reagent | Amount |
|---|---|
| Tris, pH 7.5 | 50 mM |
| NaCl | 500 mM |
| EGTA | 1 mM |
| Glycerol | 10% |
| TritonX-100 | 0.1% |
| Beta-mercaptoethanol (BME) | 0.1% |
| PMSF (from 100 mM stock) | 1 mM |

Note: BME and PMSF are added freshly when it’s time to wash.

Wash Buffer II

| Reagent | Amount |
|---|---|
| HEPES, pH 7.5 | 50 mM |
| NaCl | 100 mM |
| EGTA | 1 mM |
| Glycerol | 10% |
| Beta-mercaptoethanol (BME) | 0.1% |
| PMSF (from 100 mM stock) | 1 mM |

Note: BME and PMSF are added freshly when it’s time to wash.

Elution Buffer (4mL/protein to purify)

| Reagent | Amount |
|---|---|
| PBS, pH 8.5 | 1X |
| NaCl | 200 mM |
| TritonX-100 | 0.1% |
| Glutathione (reduced form) | 20 mM |

Note: Add Glutathione right before use.

**Procedure**

(Note: The following procedure describes the purification of one (1) yeast clone. For purifying multiple clones at the same time, follow the same procedural steps, and adjust the amount of initial buffers to prepare as needed). **The entire purification procedure is done at 4°C and with ice.**

**Cell Lysis**

1. Remove 100 mM PMSF stock solution from -20°C and heat at 65°C until crystals disappear. Mix well.
2. Prepare lysis buffer containing fresh protease inhibitors (50 ml / protein). Keep on ice.
3. Add 5 mL of zirconia beads to the 15 mL tube containing cell pellet.
(Note: DO NOT leave any beads on the screw parts of the tube and the cap!).
4. Add 5 mL of lysis buffer containing fresh protease inhibitors to the 15 mL tube. Close the cap tightly.
5. Shake the tube for 2 minutes in a paint-shaker with 2 minute resting intervals on ice.
6. Repeat step 5 a total of three times.
7. Spin at 4000 rpm for 5 minutes and transfer the supernatant to a labeled 50 ml tube.
8. Repeat steps 4 to 7 four more times for 5 times in total. The resulting supernatants are stored in the same labeled 50 mL tube.
9. Add 20 mL of lysis buffer containing fresh protease inhibitors to the 50 mL tube containing 25 mL of cell lysate.
10. Spin at 4000 rpm for 5 minutes
11. Transfer the supernatant to a new labeled 50 mL tube.

**Glutathione Bead Equilibration**

1. Add 700 µL (per protein to be purified) of Glutathione Sepharose-4B beads slurry to a labeled 50 mL tube.
2. Add 5 mL (per protein to be purified) of cold lysis buffer **without** protease inhibitors to the tube. Resuspend the beads thoroughly by pipetting.
3. Spin at 1000 rpm for 1 minute.
4. **Carefully** remove the supernatant by vacuum.
5. Repeat steps 2 to 4 two more times for a total of three (3) washes.
6. Add 5 mL (per protein to be purified) of cold lysis buffer **containing fresh protease inhibitors**. Resuspend the beads thoroughly by pipetting.

**Sample Incubation**

1. Add 5 mL of the resuspended glutathione beads to each tube containing the final cell lysates from Cell Lysis step 11.
2. Place the tube on a rotator at 4°C. Incubate for 1 hour.
3. Spin at 2000 rpm for 3 minutes.
4. **Carefully** remove the supernatant by vacuum.

**Wash I**

1. Add 5 mL of cold Wash Buffer I (with freshly added BME and PMSF) to each tube.
2. Resuspend the beads thoroughly and transfer to a 15 mL tube.
3. Spin at 2000 rpm for 3 minutes.
4. **Carefully** remove the supernatant by vacuum.
5. Add 5 mL of cold Wash Buffer I (with freshly added BME and PMSF) to each tube and resuspend the beads thoroughly.
6. Repeat steps 1 to 5 for a total of three (3) washes with Wash Buffer I.
7. **Carefully** remove the remaining supernatant by vacuum.

**Wash II**

1. Add 5 mL of cold Wash Buffer II (with freshly added BME and PMSF) to each tube and resuspend the beads thoroughly.
2. Spin at 2000 rpm for 3 minutes.
3. **Carefully** remove the supernatant by vacuum.
4. Repeat steps 1 to 3 for a total of three (3) washes with Wash Buffer II.
5. **Carefully** remove the remaining supernatant by vacuum.

**Elution**

1. Add 2 mL of Elution Buffer to each tube and resuspend the beads thoroughly.
2. Place the tube on a rotator at 4°C. Incubate rotating overnight.
3. Next morning, spin at 4000 rpm for 3 minutes.
4. **Carefully** transfer the **eluate** to a new labeled 15 mL tube.
5. Add 2 mL of Elution Buffer to each tube.
6. Place the tube on a rotator at 4°C. Incubate rotating for 1 hour.
7. Spin at 4000 rpm for 3 minutes.
8. Combine the eluate to the previously labeled 15 mL tube.

**Protein Concentration**

1. Label concentrators to be used.
2. Hydrate the concentrator membrane with 4 mL of 1X PBS.
3. Centrifuge at 4000 rpm for 10 minutes and remove the PBS.
4. Add 4 mL of eluted protein to the hydrated concentrator.
5. Spin at 4000 rpm to concentrate the purified protein to 100 µL.

**Characterization and Storage**

1. Run 1 µL of the concentrated protein on SDS-PAGE along with 0.1 µg, 0.5 µg, and 1 µg of BSA to estimate the final protein concentration.
2. Transfer concentrated protein to a labeled micro tube and store at -80°C.