

Enzyme Purification

The TA has done the following:

Streak out pUCR-TaqPol (pEJS25) onto LB Amp₁₀₀

Pick a colony into 5mL LB/AMP and grow overnight, 37C 250rpm

Inoculate 1L LB Amp₁₀₀ with the overnight culture (We have started 3 1 L cultures.)

Grow to OD₅₉₀ = 0.2 (~3-4hrs)

Add IPTG to 0.5mM (5mL 100mM IPTG per Liter)

(We have "induced" two 1 L cultures. The third culture will provide an un-induced control.)

Continue growth 16-20hours at 37 C with shaking.

Chill cells on ice

Class will start here:

Divide cells into 500 ml cultures. Each team will work with 500 ml of induced cells, and two teams will work with 500 ml of un-induced cells.

Spin cells down at 10,000 X g (~8,000 rpm in SLA1500) for 10 min 4C

Resuspend pellet in 50mL Buffer A

Spin 8K, 10min 4C

Resuspend in Buffer A plus lysozyme (12.5 ml Buffer A with 50 mg (4 mg/ml) Lysozyme added just prior to use. Incubate at RT for 15min.

Add 12.5ml Buffer B, and incubate at 75C for 40 min.

Spin cell debris down at 20,000 X g (13,000rpm in SS34) for 20min 4C

Collect supernatant (~25 ml)

Precipitate with Ammonium Sulfate:

Add (NH₄)₂SO₄ to 40% (5.35 g per 25 ml) and centrifuge at 20,000 X g (13,000rpm in SS34) for 20min 4 C.

Collect the supernatant. The pellet will float, collect supernatant by filtering through a kimwipe. Save the pellet.

Increase the (NH₄)₂SO₄ to 60% (3.03 gm per 25 ml) and pellet the 60% (NH₄)₂SO₄ cut by spinning at 34,000 X g (15,000rpm in SS34) for 30 min 4C

Resuspend the pellet in 0.5 ml Taq storage buffer

Determine the activity of the enzyme by titring it against a known standard (ie serially dilute the resuspended fraction and run PCR against a similar serial dilution of commercial Taq as the control, choose the dilution that best matches the Fisher Taq)

Solutions:

1. LB Medium (1L)

| | |
|-----------------------------------|-----------|
| Bacto Tryptone | 10.0g |
| Bacto Yeast Extract | 5.0g |
| NaCl | 5.0g |
| ddH ₂ O | to 1000mL |
| pH with 10N NaOH | to 7.0 |
| Autoclave 30min | |
| Cool media to 50C, add antibiotic | |

2. Antibiotics

Prepare Ampicillin by dissolving in water. Filter-sterilize by passing the solution through a syringe with attached 0.2um filter sterilization unit. Store at -20C. The final concentration of antibiotic in the media is dependant upon the plasmid and the host.

| Antibiotic | Solvent | Stock conc | Final Conc in Media |
|------------|------------------|------------|---------------------|
| Ampicillin | H ₂ O | 100mg/ml | 100ug/ml |

3. **PMSF** (phenylmethoysulfonyl fluoride) and **AEBSF** (4-(2-amino-ethyl)-benzenesulfonyl fluoride hydrochloride). PMSF is a VERY dangerous neurotoxin, AEBSF is a less toxic alternative (although AEBSF is STILL toxic)

| Stock | For 1mL | Final conc |
|-------------|---------|------------|
| PMSF | 17.4mg | 100mM |
| Isopropanol | 1mL | |

*PMSF becomes rapidly inactive in aqueous solutions, prepare just before use

*Use AEBSF at a concentration up to 4mM

4. Buffer A

| | 100ml | Final Conc |
|--------------------|--------|------------|
| 1M Tris (pH 7.9) | 5.0ml | 50mM |
| 25% Dextrose | 3.6ml | 50mM |
| 0.5M EDTA | 0.25ml | 1mM |
| ddH ₂ O | 91.2ml | |

5. Buffer B

| | 100ml | Final Conc |
|--------------------|--------|------------|
| 1M Tris (pH 7.9) | 1ml | 10mM |
| 1M KCl | 5ml | 50mM |
| 0.5M EDTA | 0.2ml | 1mM |
| PMSF (100mM) | 1ml | 1mM |
| Tween 20 | 500µl | 0.5% |
| Nonidet NP40 | 500µl | 0.5% |
| ddH ₂ O | 92.3ml | |

6. Taq Storage Buffer – add DTT just prior to use

| | 10ml | Final Conc |
|--------------------|--------|------------|
| 1M Tris (pH 7.9) | 0.2ml | 20mM |
| 1M KCl | 1.0ml | 100mM |
| 0.5M EDTA | 2µl | 0.1mM |
| 80% Glycerol | 6.25ml | 50% |
| 1M DTT | 10µl | 1mM |
| Nonidet NP40 | 50µl | 0.5% |
| Tween 20 | 50µl | 0.5% |
| ddH ₂ O | 2.45ml | |