Expression, purification, and assay of beta-glucosidase B

Day 1: Transform expression cells

MATERIALS

Material	Concentration	Amount per reaction
Chemically-competent *Escherichia coli* bl21(de3)		20 μL
Expression plasmid in buffer solution	> 20 ng/μL	1 μL
TB		200 μL
LB agar plates with kanamycin		1
Sterile beads		8–10
Goog tube		1

METHOD

1. Set tubes on ice and warm up 42 C dry bath
2. In an ice cold Goog tube, mix 1 μL plasmid and 20 μL competent cells

KEEP CELLS COLD AND WORK QUICKLY

3. Incubate on ice for 10 minutes
4. Incubate at 42 C for 60 seconds
5. Incubate on ice for 60 seconds
6. Add 200 μL media and recover at 37 C for 1 hour
7. Pipet 200 μL recovered cells on to plate, add glass beads, shake, and get rid of beads
8. Incubate plate overnight at 37 C

Day 2: Growth cultures

MATERIALS

Material	Amount per reaction
Transformant plates from day 1	1
Falcon tube	1
Pre-cut tube seal	1
Terrific broth with kanamycin	5 mL

METHOD

1. Aliquot 5 mL terrific broth with kanamycin into Falcon tubes
2. Inoculate tube with a single colony from plate
3. Seal tubes with tube seals
4. Incubate with shaking at **37 C** for 24 hours

Day 3: Expression cultures

MATERIALS

Material	Concentration	Amount per reaction
Growth cultures from day 2		1
Terrific broth		5 mL
IPTG	1000X	5 μL
Kanamycin	1000X	5 μL
Pre-cut tube seal		1

METHOD

1. Centrifuge growth cultures at 4,700 RPM for 10 minutes and toss supernatant
2. Make induction medium by adding IPTG and kanamycin to TB

MAKE ENOUGH FOR N+1 REACTIONS

3. Add IPTG and kanamycin to TB to make induction medium for all reactions (make N+1)
4. Add 1 mL induction medium to cell pellet in tube
5. Vortex to resuspend pellet
6. Add 4 mL induction medium to tubes
7. Seal with tube seals
8. Incubate with shaking at **18 C** for 24 hours

Day 4: Protein purification

MATERIALS

Material	Concentration	Amount per reaction
Expression culture from day 3		5 mL
Wash buffer		450 μL
Bugbuster	10X	50 μL
Dry lysis mix		1 mg
2 mL Goog tubes		1
Protein buffer		200 μL

METHOD

1. Centrifuge expression cultures at 4,700 RPM for 10 minutes and toss supernatant
2. Resuspend cell pellet in 500 μL wash buffer and transfer 1 mL to Goog tube
3. Make lysis buffer by mixing BugBuster, wash buffer, and lysis mix in a sterile tube
4. Aliquot 500 μL lysis buffer into resuspended pellets in Goog tubes
5. Rock tubes for 20 minutes
6. Centrifuge at 14,700 RPM for 10 minutes
7. Set microcolumns in rack over waste collector
8. Add 100uL of nickel resin (blue) from 50% slurry to each microcolumn
9. Add 500 μL of wash buffer and allow to drip through
10. Add 500 μL of supernatant and allow to drip through
11. Add another 500 μL of supernatant and allow to drip through
12. Wash **six times** with 500 μL of wash buffer, allow to drip through each time
13. Transfer column to fresh tube
14. Add 100 μL protein buffer directly to beads and wait five minutes
15. Add second 100 μL protein buffer and spin briefly to ensure you get all your protein
16. Read yield on the spectrophotometer and **record data** in Excel template “Yields”
17. Run a gel and set in stain on the rocker overnight

Day 5: Assay

MATERIALS

Material	Concentration	Amount per reaction
Protein solution from day 4	about 1 mg/mL	200 μL
Substrate solution	100 mM	1.8 mL
96-well plate (cheap)		1
Non-binding 96-well plate		1

METHOD

1. Remove stain from gel and replace with water
2. Perform a quick test of activity by mixing 90 μL substrate stock and 10 μL purified protein:
    1. **If this turns yellow within 5 minutes**, make 1/100 dilutions of your proteins:
        1. Add 10 μL of purified protein to 990 μL of protein buffer in a Goog tube
    2. **If this doesn't turn yellow within 5 minutes**, make 1/10 dilutions
        1. Add 100 μL of purified protein solution to 900 μL of protein buffer in a Goog tube
3. Aliquot 25 μL of diluted protein solution into a non-binding (white) plate, using three columns per mutant and three for wild type
4. In a convenient, inexpensive plate, aliquot 100 μL of substrate stock into the first row and 75 μL of protein buffer into the rest of the rows
5. With multichannel pipettor, remove 25 μL from the first row, add to second row, and mix
6. Repeat but **leave the last row alone**. Verify that every well is the same volume (75 μL)
7. Set up Gen5 program "Model enzyme kinetics"
8. Transfer 75 μL substrate from cheap plate to assay plate to initiate reaction
9. After 1 hour, examine your data to make sure the Gen5-calculated rates make sense
10. **Record your data** using Excel template "Observed rates"

Recipes

LB **plates** with kanamycin (25 plates)

Autoclave 250 mL water with 2 pellets of LB agar in a 500 mL bottle. After autoclaving, hold in 50 C dry bath until 50 C, then add 250 μL 1000X kanamycin solution. Use a serological pipet to pour 8–10 mL per plate

Lysis mix (100 reactions)

Combine 80 mg lysozyme, 10 mg DNAse, and 10 mg PMSF

Terrific broth (**TB**) (250 mL)

Dissolve 12.5 g granulated or powder TB in 250 mL water and autoclave

1 M isopropyl-beta-D-thiogalactopyranoside (1000X **IPTG**) (10 mL)

Dissolve 2.40 g IPTG in 10 mL wash buffer. Store 250 μL aliquots at –20 C

Wash buffer (1 L)

Dissolve 11.9 g HEPES, 8.7 g sodium chloride, and 0.68 g imidazole in water and adjust pH to 7.50

Protein buffer (1 L)

Dissolve 11.9 g HEPES, 8.7 g sodium chloride, and 7.31 g EDTA in water and adjust pH to 7.50

0.1 M 4-nitrophenyl-beta-D-glucopyranoside (**substrate**) (100 mL)

Dissolve 3.01 g 4-nitrophenyl-beta-D-glucopyranoside in 100 mL protein buffer. Store 1.25 mL aliquots at –20 C