SDS-PAGE

I. Sample preparation

1. Take 40 ul of bacterial overnight culture into 2 ml LB (corresponding resistance).
2. Shake bacteria fluid @ 37 C° @250rpm for 2.5 h to log phase, add inducer.
3. Shake for another 3-4 h to let the protein expressed.

* For *L.lactis*, use lysozyme for lysis:

①Add lysozyme (30 mg/ml) metal bath @42℃, 30 min.

②Ultrasonic crushing @20W for 5s and 5s pause, 60 cycles.

4. Centrifugation at 5000 rpm for 5 min, and collect the precipitated bacteria.
5. Pipette up the precipitated bacteria with residual liquid and extract into EP tube, adding 50 ul sample buffer(5X) and 200 ul 2% SDS aqueous solution.
6. Metal bath @95℃ for 30 min.

II. Gel preparation

①Separation gel

Table1. **Composition of separating gel (15%)**

Component	**Amount**
A (Separating gel buffer, pH 8.8)	4.5 mL
C (Acrylamide stock solution, 30%)	9 mL
H_2O	5.3 mL
TEMED	30L
D (AP, fresh)	100L
Total	18 mL

②Stacking Gel

Table2. **Composition of stacking gel (4%)**

Component	**Amount**

B (Stacking gel buffer, pH 6.8)	2 mL
C (Acrylamide stock solution, 30%)	1.1 mL
H_2O	5.0 mL
TEMED	20L
D (AP, fresh)	100L
Total	8.22 mL

③Running Buffer

Table3. **Composition of running buffer**

Component	**g/L**
Tris(0.025 mol/L)	3.03
Glycine(0.25 mol/L) SDS(0.1% w/v)	18.77 1.00

1. Set up the equipment for electrophoresis
2. Prepare running buffer 500mL as Table3.
3. Prepare separating gel (15%) as Table1., add TEMED just before casting.
4. Carefully cast the separating gel into the equipment, gently layer about 1 cm of water on the top of the separating gel solution to keep the gel surface flat, wait for about 5 min, suck up the water by filter paper
5. Prepare the stacking gel as Table2. and add onto the separating gel, carefully insert comb into the gel sandwich
6. Wait to allow stacking gel polymerized, add 500 mL running buffer to the electrophoresis box.
7. Remove the comb carefully.

III. SDS-PAGE

1. Load equal amounts of sample into the wells of SDS-PAGE gel (usually 8ul, adjustedto condition), 3ul of marker.
2. Run SDS-PAGE with voltage in Table4.

Table4. Voltage setting for SDS-PAGE

Stage	Voltage	Current	Time
Stage I	80V	30 mA-40mA	30 min
Stage II	120V	40 mA	50 min