

## SDS-PAGE Gel Mix

### Kit Components:

Component	5 Gels	10 Gels	20 Gels
SDS-PAGE Separating Gel Mix	50 ml	100 ml	200 ml
SDS-PAGE Stacking Gel Mix	25 ml	50 ml	100 ml
A Buffer	100 mg	100 mg	200 mg
T Buffer	100 µl	150 µl	200 µl

### Preparation

1. Each 100 mg of powder A Buffer is dissolved with 1 mL of ddH<sub>2</sub>O.
2. Aliquot 100 µL for each gel from A Buffer and store at -20 °C.

### Precautions

- It is recommended that gels are prepared fresh as the protocol for each use.
- T Buffer is volatile and it is recommended to pipette in a chemical fume hood.
- It is recommended that the T Buffer vial is parafilmmed and placed in a second sealable box after each usage.
- Nepenthe Gel-Mix is designed for mini gels (8x10cm).

### Separating Gel Preparation (for 1 Gel):

1. Wash the 1 mm spacer plate, short plate and comb with soap and brush, wipe with ethanol and dry.
2. Prepare the gel sandwich on the casting stand. Secure with clamp. Check for any leakage by placing ultrapure H<sub>2</sub>O between the two glasses and then dry.
3. Take 5 mL of SDS-PAGE Stacking Gel Mix in a falcon tube.
4. Add 50 µL of dissolved A Buffer.
5. Add 2 µL of T Buffer.
6. Mix the solution without creating bubbles and pour it between two plates.
7. Pour the solution slowly from a single point with a pipette, making sure no air bubbles are formed. Stop pouring about 1 cm from the bottom of the well.
8. Carefully add 300 µL of isopropanol to create a smooth surface on the top of the gel.
9. Wait 30-60 minutes for the polymerization.

After the polymerization, remove the isopropanol and then clean and dry it.

### Stacking Gel Preparation (for 1 Gel):

1. Take 2.5 mL of SDS-PAGE Stacking Gel Mix in a falcon tube.
2. Add 25 µL of dissolved A Buffer.
3. Add 2.5 µL of T Buffer.
4. Pour the solution slowly from a single point with a pipette, making sure no air bubbles are formed.
5. Carefully insert the combs immediately.
6. Wait 30-60 minutes for the polymerization.
7. Carefully remove the combs.

The gel is ready to use.