

PAGE-SDS Protocol (Laemmli, 1970)

| Resolving gel (LOWER) | 5% | | 7.5% | | 9% | | 10% | | 12% | | 15% | |
|--|---------------|----------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 3.4 ml per plate | 2 gels (8 ml) | 4 gels (16 ml) | 2 gels | 4 gels | 2 gels | 4 gels | 2 gels | 4 gels | 2 gels | 4 gels | 2 gels | 4 gels |
| 30% Acrylamide/BisAA (0.8%) - 37.5:1 | 1.3 ml | 2.7 | 2 ml | 4 | 2.4 ml | 4.8 | 2.7 ml | 5.3 | 3.2 ml | 6.4 | 4 ml | 8 |
| H ₂ O (milli-Q) | 4.6 ml | 9.1 | 3.9 ml | 7.8 | 3.5 ml | 7 | 3.2 ml | 6.5 | 2.7 ml | 5.4 | 1.9 ml | 3.8 |
| 1.5M Tris-HCl, pH8.8, 0.4% SDS (resolv. 4x) | 2 ml | 4 | 2 ml | 4 | 2 ml | 4 | 2 ml | 4 | 2 ml | 4 | 2 ml | 4 |
| 10% Ammonium persulfate | 80 µl | 160 | 80 µl | 160 | 80 µl | 160 | 80 µl | 160 | 80 µl | 160 | 80 µl | 160 |
| 1:10 TEMED in H ₂ O | 50 µl | 100 | 50 µl | 100 | 50 µl | 100 | 50 µl | 100 | 50 µl | 100 | 50 µl | 100 |
| Trichloroethanol (TCE) - optional | 40 µl | 80 | 40 µl | 80 | 40 µl | 80 | 40 µl | 80 | 40 µl | 80 | 40 µl | 80 |

| Stacking gel (UPPER) | 4% | |
|---|---------------|---------------|
| | 2 gels (4 ml) | 4 gels (8 ml) |
| 30% Acrylamide/BisAA (0.8%) - 37.5:1 | 0.53 ml | 1.1 |
| H ₂ O (milli-Q) | 2.3 ml | 4.6 |
| 0.5M Tris-HCl, pH6.8, 0.4% SDS (stacking 4x) | 1 ml | 2 |
| 10% Ammonium persulfate | 50 µl | 100 |
| 1:10 TEMED in H ₂ O | 30 µl | 60 |
| Trichloroethanol (TCE) - optional | 40 µl | 80 |

| 4x | Resolving | Stacking |
|------------------|------------|------------|
| Tris base | 36.33 g | 12.11 g |
| SDS | 0.8 g | 0.8 g |
| H ₂ O | to 200 ml | to 200 ml |
| pH | 8.8 | 6.8 |

| 30% AA/BisAA - 37.5:1 | |
|-----------------------|-----------|
| Acrylamide | 58.4 g |
| Bis-AA | 1.6 g |
| H ₂ O | to 200 ml |

| Coomassie Staining | |
|--------------------|------|
| Coomassie | 0.1% |
| Ethanol | 40% |
| Acetic Acid | 10% |

| 10xTris/Glycine Running Buffer w/o SDS, 2L | | |
|--|--------|--------|
| Glicine | 1.92 M | 288 g |
| Tris base | 0.25 M | 60.6 g |

| Coomassie Destain | |
|-------------------|-----|
| Ethanol | 20% |
| Acetic Acid | 10% |

To prepare 4L **1 x Running Buffer**:
40ml 10%SDS (0.1% final conc.) +
400ml 10x buffer + H₂O to 4L

| 1XRSB contains: | 4 x RSB, 10ml | |
|--------------------|-----------------|--------|
| 63 mM Tris, pH6.8; | Stacking buffer | 5 ml |
| 10% Glycerol; | Glycerol | 4 ml |
| 2% SDS; | SDS | 0.78 g |
| 0.0025% BPB | 0.5% BPB | 0.2 ml |
| 2% β-ME | β-ME | 0.8 ml |

Electrophoretic transfer of proteins (Towbin, 1979)

| WB Washing Buffer |
|------------------------|
| PBS + 0.1% Tween-20 |

| WB Blocking Buffer |
|--|
| WB Washing Buffer + 5% non-fat dry milk |

| Towbin Transfer Buffer | |
|------------------------|--------|
| Glicine | 192 mM |
| Tris base | 25 mM |
| Methanol | 20% |

| Ponceau S Staining | |
|--------------------|------|
| Ponceau S | 0.1% |
| Acetic Acid | 5% |