# DNAbiotech

**Biotechnology is our expertise**

# SDS-PAGE Gel preparation Kit

Catalog no.: DSK100

For preparation of about 40 gels

## Related products:

**Bradford Protein Assay Kit (cat#: DB0017)**

**Coomassie Brilliant Blue R-250 Staining Solution (cat#: DB9608)**

**Coomassie Brilliant Blue G-250 Staining Solution (cat#: DB9609)**

**Coomassie Brilliant Blue destining Solution (cat#: DB9610)**

**DNAbiotech Co.**

Intended for research use only

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## Introduction

The separation of macromolecules in an electric field is called *electrophoresis*. A very common method for separating proteins by electrophoresis uses a discontinuous polyacrylamide gel as a support medium and sodium dodecyl sulfate (SDS) to denature the proteins. The method is called sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). SDS (also called lauryl sulfate) is an anionic detergent, meaning that when dissolved its molecules have a net negative charge within a wide pH range. A polypeptide chain binds amounts of SDS in proportion to its relative molecular mass. The negative charges on SDS destroy most of the complex structure of proteins, and are strongly attracted toward an anode (positively-charged electrode) in an electric field. Polyacrylamide gels restrain larger molecules from migrating as fast as smaller molecules. Because the charge-to-mass ratio is nearly the same among SDS-denatured polypeptides, the final separation of proteins is dependent almost entirely on the differences in relative molecular mass of polypeptides. In a gel of uniform density the relative migration distance of a protein (Rf, the f as a subscript) is negatively proportional to the log of its mass. If proteins of known mass are run simultaneously with the unknowns, the relationship between Rf and mass can be plotted, and the masses of unknown proteins estimated.

Protein separation by SDS-PAGE can be used to estimate relative molecular mass, to determine the relative abundance of major proteins in a sample, and to determine the distribution of proteins among fractions. The purity of protein samples can be assessed and the progress of a fractionation or purification procedure can be followed. Different staining methods can be used to detect rare proteins and to learn something about their biochemical properties. Specialized techniques such as Western blotting, two-dimensional electrophoresis, and peptide mapping can be used to detect extremely scarce gene products, to find similarities among them, and to detect and separate isoenzymes of proteins.

## Storage Conditions and Reagent Preparation:

Kit will arrive packaged as a $4^{o}$ C & will performed as specified if stored as directed and used before expiration date

## Materials Supplied

| No. | Item | QTY | Storage |
|---|---|---|---|
| 1 | Acrylamide/Bisacrylamide-stock solution (30: 0~8 %) | 100 mL | 4° C |
| 2 | Separation gel buffer | 80 mL | 4° C |
| 3 | stacking gel buffer | 25 mL | 4° C |
| 4 | 5 X sample buffer | 2 × 6 mL | 4° C |
| 5 | TEMED (100 %) | 1 mL | 4° C or RT |
| 6* | Ammoniumpersulphate | 10 Calibrated tubes | 4° C or RT |
| 7 | 1X Running buffer for 1 L | 6 Packs (for 6 L) | 4° C or RT |

* Each tube contains 1 gr APS which should be reconstitute to 10% AP solution just before use. This Reagent must be used freshly.
If any of the items listed above are missing or damaged, please contact us at www.dnabiotech.ir.

## Reagent preparation

- Add 1 mL of DDW to each vial of APS Each vial of **reconstitutd APS** provides 1 mL of 10X (or 100%) APS Solution
- Dissolve the ready to use powder of SDS-PAGE running buffer (cat#: DB0016) in 1 litre of DDW. This of reconstitutd **1X** buffer can be used 2-3 times.

## Procedure

**WARNING: Unpolymerized acrylamide is a neurotoxin!**

An intact SDS PAGE electrophoresis system should include: a tank, lid with power cables, electrode assembly, casting stands, casting frames, combs (usually 10-well or 15-well), and glass plates (thickness 0.75mm or 1.0mm or 1.5mm).

(1) Clean the plates and combs. For each gel, you will need one short plate, one spacer plate, and one comb. These are usually found on the gray rack by the sink. Spray a little bit of 70% ethanol on the plates, and wipe dry with cotton. Wash the combs thoroughly with tap water. It is critical to remove all dust and small particles, especially any bits of left-over polyacrylamide.

(2) Set-up the plates on the rack. Layer the short plate on the spacer plate, with the spacers in between and slide the two plates into the green holder. Make sure that the bottom edges of the two plates are flush to avoid leakage. Lock the plates in, and place the holder on the rack, with the bottom edges of the plates pushed into the gray foam gasket to make a water-tight seal. Test the seal by pipetting or squirting a small volume of water between the plates and making sure there is no leakage. Blot dry with filter paper.

(3) **Pour the separating gel**. For each minigel (1 mm thick) you will need slightly more than 5 mL of reagent mix. Use the table below as a guide to calculate the total volumes you will need. Pipette solutions in order. Avoid introducing bubbles, which will inhibit polymerization. Swirl the

solution gently to mix thoroughly after addition of each component.

**NOTE**: The acrylamide percentage in SDS PAGE gel depends on the size of the target protein in the sample. (Details shown below)

| Acrylamide % | M.W. Range |
|---|---|
| 7% | 50 kDa - 500 kDa |
| 10% | 20 kDa - 300 kDa |
| 12% | 10 kDa - 200 kDa |
| 15% | 3 kDa - 100 kDa |

**All given amounts are in mL**

| Final Acylamide percentage (%) | 7.5 | 10 | 12.5 | 15 | 17.5 |
|---|---|---|---|---|---|
| Separation gel buffer | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Acrylamide/Bisacrylamide-stock solution | 1.45 | 1.9 | 2.45 | 2.95 | 3.4 |
| DDW | 3.05 | 2.55 | 2.05 | 1.6 | 1.1 |
| Ammoniumpersulphate (10 % or X) * | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 |
| TEMED (15 %)* | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |

(4) Pour the stacking gel. For each gel you will need about 2 mL of reagent mix. Again, pipette the solutions carefully and swirl to mix after addition of each component. Pipette the gel mix between the plates up to just below the edge of the short plate.

**All given amounts are in mL**

| Final Acylamide percentage (%) | 3 | 4 | 5 |
|---|---|---|---|
| Stacking gel buffer | 0.625 | 0.625 | 0.625 |
| Acrylamide/Bisacrylamide-stock solution | 0.25 | 0.325 | 0.41 |
| DDW | 1.6 | 1.525 | 1.45 |
| Ammoniumpersulphate (10 % or X) * | 0.04 | 0.04 | 0.04 |
| TEMED (15 %)* | 0.05 | 0.05 | 0.05 |

*** These reagents should be prepared freshly. You can either add from their stock solutions too, but the final amount should be optimized by the operator in the lab.**

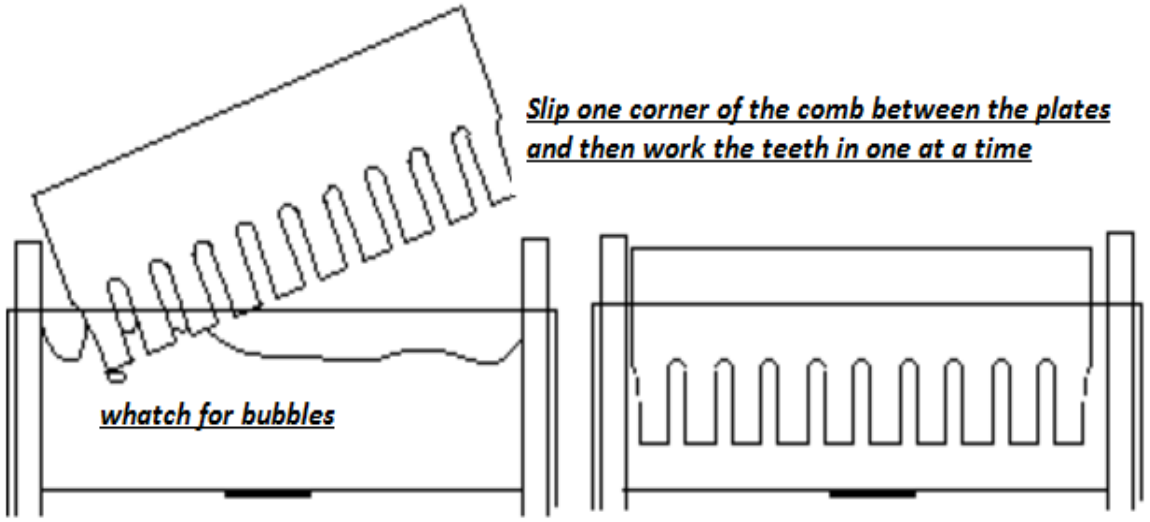
- **Once** TEMED is added, the gel will begin to polymerize, so you need to work fast (but carefully). Pipette the gel mix between the plates, making sure you leave enough space at the top for the stacking gel and comb. Carefully layer water on top of the gel solution.
- **Once** the gel has polymerized (about 10-15 mins), wash off the top of the gel with water. Carefully blot off excess water with a filter paper. Take care not to disturb or damage the top

of the gel.

- **Once** the gel has polymerized, slowly remove the comb under running water. Wash the wells carefully to avoid distorting them.

**NOTE**, **Gel storage**: Put back the comb, and sandwich the gel between two wet pieces of paper towels. The gel can be stored horizontally (with the cassette) at 4 °C for up to 1 week.

(5) Carefully place in the comb like the following image.




(6) According to your electrophoresis set assemble the cassette vertically.

(7) Pour the running buffer (adjust the volume according to the size of your tank) and bring out the comb carefully.

**NOTE**: remove the bubbles formed at the bottom of the gel.

(8) **Prepare the samples**: mix 1 volume of the sample and 4 volumes of the 5X sample loading buffer in appropriated microtube and incubate in boiling water for 5-10 minutes.

(9) Spin the microtube containing prepared samples and load appropriated volume of them into the wells.

(10) Apply electric current to the electrophoretic chamber by an authorized power supply. Set voltage to 100 - 150 volts (V) and current to 25 mA for one gel 80x8Oxl mm, 12% PAA. Stop the run after blue front dye (Bromephenole-blue) has reached bottom of the gel (after approx. 1 - 1.5 hours).

(11) Remove the gel from the electrophoretic chamber, disassemble the glass plate/ gel sandwich diligently, then fix and stain the gel tor visualization of protein bands following appropriate protocols

## Troubleshooting

| Problem | Reason | Countermeasure |
|---|---|---|
| No current | Unclosed circuit | Check contacts/leads at source of current and separation chamber; check buffer level |
| Low current | Wrong adjustment of parameters at power source | For limiting amperage select the maximum voltage recommended for the chamber; for limiting voltage select maximum amperage |
| 'Smile effect' at buffer front | Overheating | Pre-cool buffer; cooling via cooling circulator or a reduction in amperage |
| Slow migration of buffer front | Running buffer fully consumed | Always use fresh running buffer |
| Blurred bands | Diffusion after application of samples | Apply samples quickly; begin electrophoresis straight away |
| Blurred bands | Diffusion after separation | Transfer gel to fixing or staining solution immediately after electrophoresis |
| Irregular bands | Sample volumes too low or too different | Apply at least 5 µl sample; use approx. the same amounts of sample |
| Irregular bands | Differing saline content of samples | Desalinate samples as required (dialysis, gel filtration) |
| Formation of stripes | Precipitation of sample | Centrifuge or filter sample |
| Wide, partially smeared bands | Lipophilic substances in the sample | Remove substances prior to electrophoresis; increase SDS concentration if necessary |
| More bands than expected | Protease activity | Add protease inhibitor; minimize time between sample preparation and run |
| More bands than expected | Incomplete reduction | Check reduction conditions (if necessary prolong incubation time; increase DTT concentration) |

**DNAbiotech** is the 1st Iranian Biotechnology Company producing wide ranges of ready to use **molecular buffers**. These products are produced from the high quality ingredients and help researchers run their projects faster.

**Other services:**

Gene, Primer and peptide synthesizing

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