SDS-PAGE Protocol

SDS-PAGE allows an estimation of the purity of protein samples. SDS is an anionic detergent and is used to denature the proteins. The negative charges on SDS destroy most of the secondary and tertiary structure of proteins and are strongly attracted toward the anode in an electric field. Because the charge-to-mass ratio is nearly the same among SDS-denatured proteins, the final separation of proteins is almost entirely dependent on the differences in relative molecular weight (MW) of polypeptides. Some caution must be put on intrinsic strong negative or strong positively charged proteins because SDS may bind to them differently and their migration in the gel may not be at the expected MW. In PAGE the relative migration distance of a protein (Rf) is negativelyproportional to the log of its MW. To be able to estimate the MW of proteins on the SDS-page, proteins of known MW need to be run simultaneously on the gel. These proteins are called Protein Standards (MB-201-0200).

Beside the protein MW marker Rockland also offers a 10% SDS solution (MB-015), SDS-PAGE running buffer (MB-017) and sample loading buffer (MB-018).

Procedure

1. Decide which percentage of gel you need to separate your proteins Eg.:
   Use 4–8% gels to separate proteins 100–500 kDa in size.
   Use 4–20% gels to separate proteins 10–200 kDa in size.
2. Place your gel in a clean plastic electrophoresis chamber and corresponding gel holder.
3. Prepare 1X SDS-PAGE Running Buffer as follows: for 500 mL of 1X SDS-PAGE Running Buffer by adding 50 mL of 10X SDS-PAGE Running Buffer (MB-017) to 450 mL of diH_20 (MB-009-1000).
4. Fill the inner portion between the gel(s) and the gel holder with the appropriate 1X Running Buffer. Pour the remaining 1X Running Buffer into the outer chamber.
5. Sample Preparation:
   If using a pre-prepared lysate (already in sample buffer), thaw lysate and transfer 25 µL of lysate to a clean pre-labeled microcentrifuge tube. Add β-mercaptoethanol (BME) to a final concentration of 0.55M, i.e., add 1 µL stock BME per 25 µL lysate. Mix well by pipetting. Label microcentrifuge tubes with sample description, volume, and concentration of lysate.

   Any other protein samples: transfer to clean pre-labeled microcentrifuge tubes and mixed with an equal volume of 2X Sample Buffer (MB-018) with 0.55 M BME. Sample protein concentration should be sufficiently high, eg.: final protein concentrations from 1 µg to 500 µg depending on protein type and detection method.

   Prepare MW standards (MB-201-0200) for electrophoresis. For SDS-PAGE use either an unstained MW standards or pre-stained MW marker. For SDS-PAGE followed by western blotting, use pre-stained MW markers.

   Record lane number, sample description, sample concentration, loading volume, loading amount and addition of reducing agent for all samples.
6. Place all microcentrifuge tubes containing samples for SDS-PAGE into a heating block (set to 95°C) or water bath. Heat samples for 5 minutes.
7. After heating, centrifuge the aliquots for 3 minutes using a microcentrifuge to pellet any debris.
8. Load all samples into gel lanes starting with the MW standards. Sample loading volumes should be from 5 µL–35 µL per lane (depending on gel). If protein concentrations are from 100 µg/mL–500 µg/mL, then sample amounts will range from 0.5µg–17.5 µg per lane. Generally, 1.0 µg is sufficient to visualize purified proteins and 10 µg is sufficient to visualize proteins in lysates on a Coomassie stained gel.
9. Cover the chamber and firmly connect both the anode and the cathode. Set the voltage on the electrophoresis power supply to a constant voltage of 150V. Turn ON the power supply.
   CAUTION! Do not touch the electrophoresis unit while power is on. If buffer is leaking from the unit be certain to turn power OFF before making contact with buffer. Use care at all times.
10. Allow the gel to electrophorese for 45–90 minutes. Turn OFF the power immediately after the dye front migrates out from the bottom of the gel.
11. Disconnect the electrodes and remove the cover. Remove gel holder from the electrophoresis chamber. Carefully remove the gel from holder. Remove the gel from its plates and proceed with desired detection method.