Technische Universiteit
Eindhoven
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iGEM TU/e 2017
Biomedical Engineering

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SDS-PAGE Analysis

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1 SDS-Page Analysis

1.1 Preparation of reagents

Estimated bench time: 30 minutes
Estimated total time: 30 minutes
Purpose: Preparation of buffers and solutions.

1.1.1 Materials

- Balance
- Bottle (glassware)
- Bromophonol blue
- dH_2O
- ddH_2O
- Glycerol
- Glycine
- DTT
- SDS
- Tris base
- Tris-HCl
- β-mercaptoethanol

1.1.2 Setup & Protocol

- For 1 M DTT, the correct amounts are:
  - 2.32 g DTT
  - 15 ml dH_2O
- For 2X sample buffer SDS-PAGE, the correct amounts are:
  - 1 ml 1M Tris-HCl, pH 6.8
  - 10 ml dH_2O
  - 2 ml glycerol
  - 2.5 ml β-mercaptoethanol
  - 4 ml 10% SDS
  - 500 µl 1% Bromophonol blue
- For 2X Laemmili buffer, the correct amounts are:
  - 450 µl of 2X sample buffer SDS-PAGE
  - 50 µl 1M DTT
- For 1X SDS running buffer, the correct amounts are:
  - 1.8 l ddH_2O
  - 2 g SDS
  - 28.8 g glycine
  - 6.04 g Tris base

1.2 Sample preparation

Estimated bench time: 20 minutes
Estimated total time: 45 minutes
Purpose: Preparing samples for loading on the SDS-PAGE gel.

1.2.1 Materials

- 10 µl 2X Laemmili buffer
- 2X Laemmili buffer
- BugBuster™ Protein Extraction Reagent
- Culture tube with cells and expressed proteins
- DTT stock (1M)
- Eppendorf tubes
- Heat/shaking block
- MiniSpin Centrifuge
- Tabletop Centrifuge
- Thermal cycler
- Vortex

1.2.2 Setup & Protocol

- Spin down the culture tube with cells and expressed proteins for 15 minutes at 3000 xg.
- Discard supernatant.
- Add BugBuster™ Protein Extraction Reagent. This needs to be 1/50th volume of the original volume of the culture.
- Shake at 250 rpm at room temperature until the pellet is dissolved.
- Spin down for 10 minutes at 13,400 rpm.
- Transfer the supernatant to a new Eppendorf tube.
- Mix 10 µl sample with 10 µl 2X Laemmili buffer in a PCR tube.
- Mix by vortexing and short-spin to get the samples at the bottom of the tubes.
- Denaturate for 5 minutes at 95 °C in the thermal cycler.

1.3 Preparation of the gel

Estimated bench time: 10 minutes
Estimated total time: 10 minutes
Purpose: Preparing a SDS-PAGE gel (10%) for loading the samples.

1.3.1 Materials

- 1X SDS running buffer
- Precasted SDS-PAGE gel (10%)
- SDS-PAGE kit components:
  - Cell for SDS-PAGE gels
  - SDS-PAGE casting tray
  - SDS-PAGE dummy

1.3.2 Setup & Protocol

- Take a cell for SDS-PAGE gels and fill this with 1X SDS running buffer for 50%.
- Take a precasted gel (10%) and take it out of the packing. Remove the strip at the bottom of the gel.
- Take a casting tray and clamp the gel (in the right direction!) and the dummy in this tray.
- Fill the chamber between the gel and dummy completely with 1X SDS running buffer.
- Make sure the chamber is not leaky.

1.4 Running of the gel

Estimated bench time: 45 minutes
Estimated total time: 5 minutes
Purpose: Analysis of the protein product with a SDS-PAGE gel.

1.4.1 Materials

- Extra-long pipet points
- Precision Plus Protein™ Dual color protein ladder
- Prepared loading samples
- Precasted SDS-PAGE gel (10%)

1.4.2 Setup & Protocol

- Load 10 µl of the prepared loading samples on the gel with extra-long pipet points.
- Load 10 µl of Precision Plus Protein™ Dual color protein ladder on the gel.
- Run the gel for 40 minutes on 200 V.

1.5 Staining of the gel

Estimated bench time: 20 minutes
Estimated total time: 2.5 hours
Purpose: Analysis of the protein product with a SDS-PAGE gel.

It is essential to work with gloves when working with the staining reagents used.

1.5.1 Materials

- Bio-Safe™ Coomassie G-250 stain
- dH_2O
- ImageQuant
- Rocking plate
- Tray (for transporting the SDS-PAGE gel)
- Tin foil

1.5.2 Setup & Protocol

- Carefully take the gel out of the casting frame and put it in a tray.
- Wash the gel with a layer of dH_2O for 10 minutes on a rocking plate. Cover the tray with tin foil.
- Stain the gel with a layer of Bio-Safe™ Coomassie G-250 stain for 1 hour on a rocking plate. Again, cover the tray with tin foil.
- Discard the Bio-Safe™ Coomassie G-250 stain in the sink.
- Destain the gel with a layer of dH_2O for 1 hour on a rocking plate.
- Create an image of the gel using the ImageQuant.