SCyCode – PROTOCOL

1D SDS-PAGE purification of low complexity samples (vers.1)

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MATERIALS

- NuPAGE pre-cast 12% Bis-Tris Gel (1.0 mm * 10 wells; Invitrogen NP0341)
- NuPAGE LDS Sample Buffer (4x concentrated; Invitrogen NP0008)
- NuPAGE MOPS SDS Running buffer (20x concentrated; Invitrogen NP0001-02)
- InstantBlue™ Ultrafast Protein Stain (▲ HAZARDS)

EQUIPMENT

- XCell SureLock Mini-Cell Electrophoresis System (novex, life technologies)

PROCEDURE

Purification of low complexity protein samples (e.g. from immunoprecipitation) for subsequent in-gel digestion and LC-MS/MS analysis. Therefore, sample proteins are transferred approx. 1 cm into a polyacrylamide gel in a short, non-separating 1D SDS-PAGE.

Short 1D SDS-PAGE

1. Samples (max. 100 µg protein per well) are mixed with 4x NuPAGE LDS Sample Buffer (25% v/v of the final volume) and β-Mercaptoethanol (10% v/v of the final volume); **NOTES[1,2]**
   A maximum of 45 µL can be loaded per well; **NOTE[3]**
2. 800 mL 1x MOPS SDS Running buffer are prepared with de-ionized water.
3. Pre-cast NuPAGE gel is removed from wrapping, white cover tape on the gel casing anode side is removed and the gel is rinsed with deionized water.
4. Gel is inserted in XCell chamber and inner chamber is filled with 1x MOPS SDS Running buffer, tightness is monitored.
5. Samples are loaded into wells and 1x LDS Sample Buffer (diluted in MilliQ water) is loaded in surrounding wells (equal volume as sample volume); see **NOTE[3]**
6. Outer chamber is filled with 1x LDS Sample Buffer.
7. Gel-electrophoresis for approx. 10 min with 200V constant, until dye front reaches 1.0 - 1.5 cm into the gel.

GEL STAINING and BAND CUTTING

8. Gel is removed from plastic casing and stained for 60 min with InstantBlue.
9. After protein staining, gel is rinsed with MilliQ water and sample protein band is cut out with a scalpel.
10. Storage of the gel piece in a sample tube at 4 °C (for several days) upon subsequent in-gel digestion.
11. Continue with In-gel digestion protocol

NOTES

NOTE [1] - step 1: High concentrated samples can be diluted with MilliQ water.

NOTE [2] - step 1: No heating of the samples is necessary.

NOTE [3] - steps 1&5: If multiple samples are loaded onto one gel, always leave one well empty between sample (loaded with 1x Sample Buffer) to avoid cross contamination; careful pipetting with gel loader tips is crucial.

HAZARDS

Substance/Buffer	**Hazardous Component**								
Instant blue			+						