# Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis (SDS-PAGE) with Sypro straining

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## Abstract

This protocol provides an efficient way to observe proteins in samples.

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## Before start

Prepare acrylamide gels a few hours before running the gels; acrylamide percentage depends on protein sizes. Prepare SDS-PAGE buffers beforehand as well.

## Protocol

### Protein sample preparation

**Step 1.**

Load proteins with loading buffer (containing loading dye) in a 3:1 ratio in Eppendorf tubes, then boil samples for 5 min at 100°C.

### Protein migration by electrophoresis

**Step 2.**

Load 20 µl of protein samples and a protein standard molecular weight marker in gels placed in a vertical gel tank system filled with 1x running buffer. Run gels at 100 volts for 2 hours at room temperature.

AMOUNT

20 µl Additional info: Protein samples

### Protein fixation on gel

**Step 3.**

After electrophoresis, immerse gels in a fixing solution bath for 30 min on a rotary shaker. (fixation 1/2)

Protein fixation on gel

**Step 4.**

Repeat the fixation one more time; immerse gels in a fixing solution bath for 30 min on a rotary shaker. (fixation 2/2)

Protein staining with SYPRO solution

**Step 5.**

Immerse gels in 40 ml of pre-diluted SYPRO solution and incubate overnight, protected from the light on a rotary shaker at room temperature.

AMOUNT

40 ml Additional info: Pre-diluted SYPRO

Protein wash with washing buffer

**Step 6.**

Wash gels with a washing solution for 30 min on a rotary shaker at room temperature, in a container protecting gels from the light.

SAFETY INFORMATION

**Collect SYPRO solution in a specific container waste and follow MSDS and health and safety regulation to discard.**

Protein wash with ultrapure water

**Step 7.**

Immerse gels in ultrapure water and wash for 5 min at room temperature on a rotary shaker, in a container protecting gels from the light. (wash 1/2)

SAFETY INFORMATION

**Collect washing buffer in a properly labeled container and follow health and safety regulation to discard.**

Protein wash with ultrapure water

**Step 8.**

Immerse gels in ultrapure water and wash for 5 min at room temperature on a rotary shaker, in a container protecting gels from the light. (wash 2/2)

SAFETY INFORMATION

**Collect washing buffer in a properly labeled container and follow health and safety regulation to discard.**

Reading gels

**Step 9.**

Read gels under UV machine.

SAFETY INFORMATION

**Wear required protecting gear and discard gels in a acrylamide-specific container to be discarded following health and safety regulation.**

## Warnings

Acrylamide is carcinogenic and should be handled and disposed of accordingly to MSDS sheet and Health and Safety regulation.