Electrophoretic Mobility Shift Assay (EMSA) Kit (E33075)

Quick Facts

Storage upon receipt:

- ≤–20°C
- Desiccate
- Protect from light

Ex/Em:

- 255, 495/520 nm for SYBR® Green EMSA stain, bound to DNA or RNA
- 280, 450/610 nm for SYPRO® Ruby EMSA protein gel stain

Number of assays: 10 minigels

Introduction

Molecular Probes' fluorescence-based Electrophoretic Mobility Shift Assay (EMSA) Kit provides a fast, easy, and quantitative method to detect both nucleic acid and protein in the same gel, doubling the information that can be obtained from electrophoretic mobility shift assays. This kit uses two fluorescent dyes for detection — SYBR® Green EMSA nucleic acid gel stain for RNA or DNA and SYPRO® Ruby EMSA protein gel stain for proteins. The nucleic acids and proteins are stained in the gel after electrophoresis. There is no need to pre-label the DNA or RNA with a radioisotope, biotin, or a fluorescent dye before the binding reaction, hence there is no possibility that the label will interfere with protein binding. Staining for nucleic acid only takes about 20 minutes, and about 4 hours for the subsequent protein staining. Results can be obtained much faster than with radioisotope labeling, which may require multiple exposure times, or with chemiluminescence-based detection, which requires blotting and multiple incubation steps. Using the two stains provided in the kit makes it possible to obtain quantitative information on both the nucleic acid (down to 1 ng) and the protein (down to ~30 ng). Because the signal from the stains is linear over a broad range, the amount of nucleic acid and protein can be determined accurately, even in a single band. Both stains can be detected using a standard 300 nm UV transilluminator, a 254 nm UV epi-illuminator or a laser-based scanner (Figure 1, panels A and B). Digital images can be easily overlaid for a two-color representation of nucleic acid and protein in the gel.

Materials

Contents

- **SYBR® Green EMSA nucleic acid gel stain** (Component A), 100 µL of a 10,000X concentrate in dimethylsulfoxide (DMSO)
- **SYPRO® Ruby EMSA protein gel stain** (Component B), 650 mL of an aqueous 1X solution
- **Trichloroacetic acid** (TCA, Component C), 87.5 g
- **6X EMSA gel-loading solution** (Component D), 1 mL
- **5X binding buffer** (Component E), 200 µL

Each kit contains sufficient reagents to stain ten 6 cm × 9 cm × 0.75 mm minigels.

Storage

Upon receipt, store the kit at ≤–20°C, desiccated, and protected from light. When stored properly, the components of the kit should be stable for at least 6 months.

Handling

WARNING: TCA (Component C) is very corrosive and can burn the skin — use with caution.

No data are available regarding the toxicity of SYBR® Green EMSA nucleic acid gel stain (Component A) and SYPRO® Ruby EMSA protein gel stain (Component B). Because the SYBR® Green EMSA stain binds to nucleic acids, it should be treated as a potential mutagen and therefore handled and disposed of with appropriate care and in accordance with all applicable regulations. The DMSO stock solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. The SYPRO® Ruby EMSA protein gel stain comprises an organic component and a heavy metal component (ruthenium).

Spectral Characteristics

SYBR® Green EMSA stain is maximally excited at 495 nm but has a secondary excitation peak at ~255 nm. The fluorescence emission of SYBR® Green EMSA stain bound to nucleic acid is centered at 520 nm. SYPRO® Ruby EMSA stain is maximally excited at 450 nm but has a secondary excitation peak at ~280 nm. The fluorescence emission of SYPRO® Ruby EMSA stain bound to protein is centered at ~610 nm. These spectral characteristics make both stains compatible with a wide variety of gel imaging systems, ranging from conventional photography using UV epi- or transillumination to sophisticated scanning instruments.

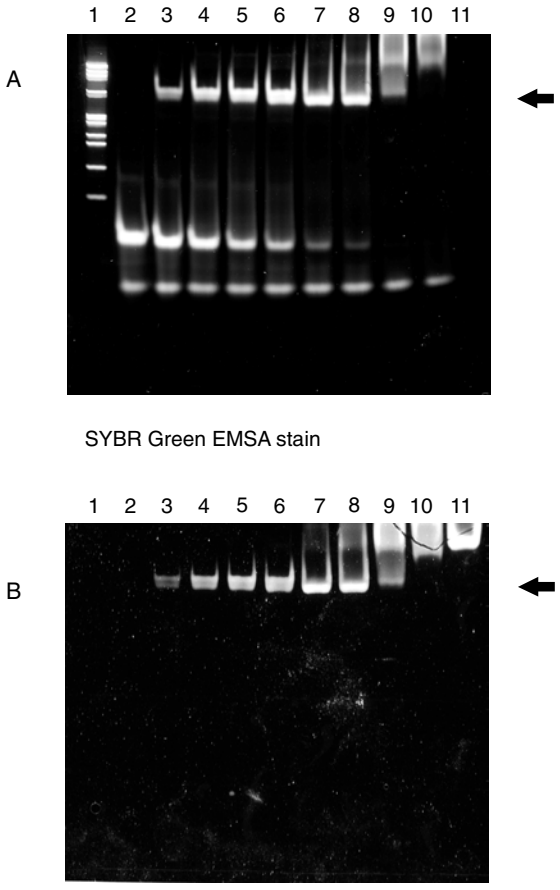

Figure 1. Titration of *lac* operator DNA with *lac* repressor protein. Increasing amounts of *lac* repressor protein (monomer molecular weight 37,500 daltons) were added to *lac* operator DNA in a final 10 µL volume of 1X *lac* repressor:operator binding buffer. Each sample was incubated for 20 minutes before loading onto a 6% nondenaturing polyacrylamide gel. The gel was run for 35 minutes at 200 V in pre-chilled 0.5X TBE. Lane 1: DNA markers (ΦX174 DNA cut with *Hae* III). Lane 2: *lac* operator DNA only (40 ng). Lane 3–10: *lac* operator DNA in 40 ng aliquots with increasing amounts of *lac* repressor protein (65, 130, 195, 260, 390, 520, 780, 1040 ng). Lane 11: *lac* repressor protein only (1040 ng). The gel shown in panel A was stained with SYBR® Green EMSA stain. The gel shown in panel B is the same gel stained with SYPRO® Ruby EMSA stain. The arrow indicates the *lac* repressor:operator complex, which is stained in both panels.

Preparation of Samples and Controls

1.1 Prepare the controls. Thaw Component E completely, keeping the vial on ice. Prepare the binding reaction for the control DNA and protein as follows:

- 2 µL of deionized water (dH_2O)
- 2 µL 5X binding buffer (Component E)

Mix gently but thoroughly, and incubate the reaction for 20 minutes at room temperature.

1.2 Prepare the samples. Prepare the binding reactions for your DNA or RNA fragment and protein. In addition, prepare control reactions of the fragment alone and the protein alone. Use a binding buffer and incubation time appropriate for your protein. The 5X binding buffer included in the kit (Component E) is 750 mM KCl, 0.5 mM dithiothreitol, 0.5 mM EDTA, 50 mM Tris, pH 7.4 and is optimized for *lac* repressor binding to *lac* operator. This provided buffer may not be optimal for other protein–nucleic acid interactions. There is no need to pre-label either the nucleic acid or the protein. Note that high molecular weight dI-dC should not be used in the binding reaction as this will stain with the SYBR® Green EMSA stain and may obscure the gel-shift band.

1.3 Separate the reaction mixture by nondenaturing polyacrylamide gel electrophoresis. At the end of the incubation period, add 2 µL of 6X EMSA gel-loading solution (Component D) for each 10 µL of reaction mixture, and mix gently but thoroughly. Separate the DNA– or RNA–protein complexes by electrophoresis using a nondenaturing polyacrylamide gel.

Staining Nucleic Acids with SYBR® Green EMSA Nucleic Acid Gel Stain

The following protocol is optimized for 6 cm × 9 cm × 0.75 mm minigels. Larger gels will require proportionally larger volumes. After staining with the SYBR® Green EMSA stain, the gel must be imaged and documented before staining for protein with the SYPRO® Ruby EMSA stain.

2.1 Thaw the SYBR® Green EMSA gel stain concentrate. *Before opening the vial*, warm the vial of SYBR® Green EMSA nucleic acid gel stain (Component A) to room temperature, vortex mix the contents to ensure homogeneity and centrifuge the vial briefly to bring the contents to the bottom of vial.

2.2 Dilute the SYBR® Green EMSA gel stain concentrate. SYBR® Green EMSA gel stain is best stored as it is provided, i.e., as a 10,000X concentrate; therefore, dilute only the required amount for the day's experiment. For a typical minigel, dilute 5 µL of the 10,000X concentrate (Component A) into 50 mL of TBE buffer (not provided; 89 mM Tris base, 89 mM boric acid, 1 mM EDTA, pH ~8.0).

2.3 Stain the gel. Place the gel in a clean plastic staining container such as a plastic weigh boat or the top of a pipet-tip box. Add sufficient 1X SYBR® Green EMSA staining solution (prepared in step 2.2) to cover the gel, about 50 mL for a 6 cm × 9 cm × 0.75 mm gel. Incubate the gel in the 1X stain with continuous, gentle agitation (e.g., on an orbital shaker at 50 rpm) for ~20 minutes, protected from light.

- Do not use glass dishes, as these may adsorb the dye.
- The optimal staining time may vary depending on the thickness of the gel and the percentage of polyacrylamide.
- Do not reuse the staining solution as this will result in a significant loss of sensitivity.
- Any unused 1X staining solution may be stored for later use for up to 7 days *in a plastic container* at 2–6°C, protected from light.

2.4 Wash the gel. Wash the gel in 150 mL of dH_2O for ~10 seconds to remove excess stain that may interfere with image analysis. Repeat the wash once for a total of two washes.

2.5 Visualize the stained nucleic acids and document the image. For conventional or digital photography, use 300 nm UV transillumination or 254 nm UV epi-illumination. For optimal sensitivity, appropriate photographic filters must be used; e.g., for a Polaroid camera and black-and-white print film use a 490 nm longpass filter such as the SYBR® photographic filter (S7569) or the SYPRO® photographic filter (S6656). The red-orange filters typically used to photograph gels stained with ethidium bromide

will not work well. For other cameras, such as a CCD camera, use a 520 nm bandpass filter, which corresponds with the emission characteristics of the dye. For laser-based scanners use an instrument that excites at 450, 473 or 488 nm, and use parameters and filter sets appropriate for visualizing fluorescein (FITC), SYBR® Green I, SYBR® Green II or Alexa Fluor® 488 dyes. See also *General Tips for Visualizing Fluorescent Bands in Gels*, below.

Staining the Proteins

The following protocol is optimized for 6 cm × 9 cm × 0.75 mm minigels. Larger gels will require proportionally larger volumes. Incubation with SYPRO® Ruby EMSA protein gel stain will wash away the SYBR® Green EMSA nucleic acid gel stain. Therefore, gels stained with SYBR® Green EMSA stain should be imaged before proceeding with protein staining.

3.1 Add TCA to the SYPRO® Ruby EMSA protein gel stain. The working solution of SYPRO® Ruby EMSA stain must contain trichloroacetic acid (TCA). To make this working solution, dissolve the provided TCA (Component C) in the provided solution of SYPRO® Ruby EMSA stain (Component B) as described below. **WARNING:** TCA is highly corrosive and hazardous; use appropriate precautions such as eye protection, gloves, lab coats, etc., when working with solid TCA or any TCA-containing solution.

- Pour ~100 mL of the thawed SYPRO® Ruby EMSA protein gel stain (Component B) into the bottle containing the TCA (Component C). The TCA bottle should be slightly over half full.
- Replace the cap securely on the TCA bottle, and carefully shake the contents to dissolve the TCA, which should take about 5 minutes.
- Pour the TCA solution back into the bottle containing the rest of the SYPRO® Ruby EMSA stain.
- Replace the cap securely on the SYPRO® Ruby EMSA stain bottle, which now contains TCA, and mix carefully by inverting the bottle at least ten times.
- Put a check mark in the box on the Component B label to indicate that TCA has been added.
- Store the SYPRO® Ruby EMSA protein gel stain with TCA at room temperature, protected from light. When stored properly, the solution should be stable for at least 6 months.

3.2 Stain the gel for 3 hours in the dark. Place the gel in a clean plastic staining container such as a plastic weigh boat or the top of a pipet-tip box. Add sufficient SYPRO® Ruby EMSA protein gel stain with TCA (prepared in step 3.1) to cover the gel; about 50 mL for a 6 cm 9 cm 0.75 mm gel should be sufficient. Incubate the gel with continuous, gentle agitation (e.g., on an orbital shaker at 50 rpm) for ~3 hours in the dark.

- Do not use glass dishes, as these may adsorb the dye.
- For convenience, gels may be left in the dye solution overnight or longer without overstaining.
- Do not dilute the stain, as diluted stain will result in decreased sensitivity.
- Do not reuse the staining solution as this will result in a significant loss of sensitivity.

3.3 Wash the gel in water. Wash the gel in 150 mL of dH_2O for ~10 seconds. Repeat the wash once for a total of two washes.

3.4 Destain the gel in 10% methanol, 7% acetic acid. Wash the gel in 10% methanol, 7% acetic acid for 60 minutes. A gel stained overnight may require a longer destaining.

3.5 Wash the gel in water. Wash the gel in 150 mL of dH_2O for ~10 seconds. Repeat the wash once for a total of two washes.

3.6 Visualize the stained protein and document the image. For conventional or digital photography, use 300 nm UV transillumination or 254 nm UV epi-illumination. For optimal sensitivity, appropriate photographic filters must be used; e.g., for a Polaroid camera and black-and-white print film, use a 490 nm longpass filter such as the SYPRO® photographic filter (S6656). The red-orange filters typically used to photograph gels stained with ethidium bromide will not work well. For other cameras, use a filter that corresponds as closely as possible with the emission characteristics of the dye. For laser-based scanners, use an instrument that excites at 450, 473 or 488 nm and use parameters and filter sets appropriate for visualizing Molecular Probes' SYPRO® Ruby protein gel stain (S12000, S12001, S21900). For information on appropriate filter sets to use with common laser-based scanners, see the reference table in our product information sheet, *Imaging Platforms for Visualization of SYPRO® Ruby Protein Stains*, which is available at www.probes.com. See also *General Tips for Visualizing Fluorescent Bands in Gels,* below.

General Tips for Visualization of Fluorescent Bands in Gels

- It is important to clean the surface of the imaging bed after each use with dH_2O and a soft cloth (like cheesecloth). Otherwise, fluorescent dyes can accumulate on the glass surface and cause high background fluorescence.
- To obtain the maximal sensitivity from 300 nm UV transillumination, use a transilluminator with six 15-watt bulbs. Excitation with less powerful light sources may give less sensitivity.
- Using a CCD camera, images are best obtained by digitizing at about 1024 1024 pixels resolution with 12- or 16-bit gray scale levels per pixel. Please contact your camera manufacturer for recommendations on filter sets. A CCD camera–based image analysis system can gather quantitative information that will allow comparison of fluorescence intensities between different bands or spots.
- The polyester backing on some premade gels is highly fluorescent. For maximum sensitivity using a UV transilluminator, the gel should be placed polyacrylamide side down and an emission filter, such as the SYPRO® photographic filter (S6656), used to screen out the blue fluorescence of the plastic. Using a laser scanner will reduce the amount of fluorescence from the plastic backing so that the gel may be placed polyester side down.

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
E33075	Electrophoretic Mobility-Shift Assay (EMSA) Kit *with SYBR® Green and SYPRO® Ruby EMSA stains* *10 minigel assays*	1 kit

Contact Information

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Please visit our website — **probes.invitrogen.com** — for the most up-to-date information.

Molecular Probes, Inc.
29851 Willow Creek Road, Eugene, OR 97402
Phone: (541) 465-8300 • Fax: (541) 335-0504

Customer Service: 6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338 • Fax: (541) 335-0305 • probesorder@invitrogen.com

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Phone: (541) 335-0353 • Toll-Free (800) 438-2209
Fax: (541) 335-0238 • probestech@invitrogen.com

Invitrogen European Headquarters

Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100 • Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

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