

## EMSA Frequently Asked Questions

Are reagents in the EMSA kit sold separately?	Yes, reagents used in the EMSA kit are sold separately. You can see a list of these reagents on our by clicking on the Catalog Listings tab on this webpage: <a href="http://www.panomics.com/product.php?product_id=21">http://www.panomics.com/product.php?product_id=21</a> .
Bands do not appear distinct or background within well is too high	1) Verify that there is absolutely no SDS contamination in the gel apparatus, plates, etc. Trace amounts of SDS will cause the protein-DNA complexes to separate during the running of the gel, resulting in a smear. 2) The temperature needs to be maintained at 4 C or lower, since heat will be generated during the running of the gel and may cause melting of the gel and resulting poor transfer to the membrane.
Can a semi-dry transfer method be used instead of the wet transfer method?	Yes, semi-dry transfer method is also compatible. Any western blotting transfer method is suitable for EMSA.
Can I order just the EMSA Kit without probe?	Yes, you can place an order for AY1000, which is the EMSA Kit without Probe. This kit contains the reagents for running 5, 8x10 gels. It is the same kit that is included with EMSA Combo Kit (AY9999) and the EMSA Kit with Probe (AYxxxxP).
Can I order the probe only?	Yes, the probe only can be ordered by placing a "P" at the end of the catalog number. For example, for NFkB, AY1030P will include only the labelled probe and cold probe, but AY1030 will include the EMSA Kit components as well as the labelled probe and cold probe.
Can I use any positively charged nylon membrane?	In theory any positively charged nylon membrane will work for the transfer. However, we have only tested the Biodyne® B, (P/N 60201 from Pall) membrane, and therefore we recommend its use.
Can I use other chemiluminescent substrates for detection?	It is possible to use other HRP compatible chemiluminescent substrates for detection. However, we recommend the use of Panomics substrate with our EMSA Kits. This is because results with other substrates may vary depending upon the quality or characteristic of the chemiluminescent substrate used.

<p>Can the EMSA kit be used to perform supershifts as well?</p>	<p>Yes, our EMSA kits can be used for supershift assays as well. However, we do not provide the reagents for this assay. Panomics offers Transcription Factor ELISAs as an alternative to supershift assays. This kit allows for the antibody-based detection of DNA-bound transcription factors in a 96-well plate format. For more information on Transcription Factor ELISAs, please go to <a href="https://www.panomics.com/product.php?product_id=23">https://www.panomics.com/product.php?product_id=23</a>.</p>
<p>Does increasing the volume of nuclear extract affect the results?</p>	<p>No, the increase volume in nuclear extract and subsequent decrease in water does not affect results because the binding buffer helps compensate for the difference in salt concentration.</p>
<p>How are the EMSA probes labeled? Are the EMSA probes single- or double-stranded?</p>	<p>The EMSA probes are double-stranded. These probes are biotin-labeled on the 5' end on the sense strand.</p>
<p>How specific are the probes for each transcription factor?</p>	<p>Transcription factors belonging to the same family will bind to the probe sequence. A supershift assay or TF ELISA should be performed using an antibody specific to the transcription factor of interest. Panomics does not provide kits for the supershift assay; however we offer Transcription Factor ELISAs as an alternative to supershift assays. This kit allows for the antibody-based detection of DNA-bound transcription factors in a 96-well plate format. For more information on Transcription Factor ELISAs, please go to <a href="https://www.panomics.com/product.php?product_id=23">https://www.panomics.com/product.php?product_id=23</a>.</p>
<p>I do not see any shifted bands with my nuclear extract sample.</p>	<p>Please check the following to troubleshoot this problem: 1) What is the concentration of nuclear extract being loaded. Check to see if it is in acceptable range of at least 1-2 ug loaded per reaction. 2) Verify that transfer is occurring --- is the blue loading dye transferred over to the membrane? 3) Check to see if the free probe is present on the EMSA image. If not present, it is possible that the free probe ran off the gel. Free probe should always be present. The gel should be run at the 120 Volts for 50- 55 minutes or until the blue dye reaches 1 cm from the bottom. If these criteria are in place, it is likely that there was a problem with the transfer itself. 4) It is important to verify that there is absolutely no SDS contamination in the gel apparatus, plates, etc. Trace amounts of SDS will cause the protein-DNA complexes to separate during the running of the gel, resulting in a smear. 5) The electrophoresis temperature needs to be maintained at 4 C or lower, since heat will be generated during the running of the gel and may cause melting of the gel and resulting poor transfer to the membrane.</p>

Is it ok to use pre-cast gels for EMSA?	Many customers do use pre-cast gels with our EMSA kits. However, Panomics has not tested other vendors pre-cast gels - we only recommends using gels made in-house. This is because the pre-cast gels tend to be thicker and may cause problems during the transfer step.
Is it possible to use PVDF or nitrocellulose membranes for EMSA?	Nylon membranes are recommended for use with our EMSA kits. Nitrocellulose membranes are typically used with proteins which is not ideal with EMSA since an EMSA gel has both proteins and oligonucleotides on it. PVDF strongly binds to protein, but weakly binds to DNA - so it is also not suitable for use during the EMSA. We recommend the use of Biodyne® B, (P/N 60201 from Pall), membranes with our kit.
Is there a recommended supershift protocol?	We do not have a recommended supershift protocol. However, here is a simple version of a supershift protocol that was previously used here at Panomics (attached).
There is nothing detected on my membrane - the membrane is completely blank.	It is likely that the transfer was not done correctly. 1) Verify that the sandwich in the electroblotting device was arranged properly. Sometimes the electrodes are reversed causing the DNA and protein in the sample to migrate away from the membrane. 2) Check to see if the loading dye transferred to the membrane after the transfer. 3) Check to see what kind of membrane was used. We recommend the use of the Biodyne B Nylon Membrane (P/N 60201 from Pall)
What are the controls provided with the EMSA kit?	The control nuclear extract, control probe, and cold control probe allow you to assess the running of the gel, transfer, and detection steps of the protocol. The control extract provided is HeLa nuclear extract and the control probe is the Ets-1 probe sequence.
What are the EMSA probes diluted in?	The EMSA probes are diluted in dH2O and should be stored at -20 C.
What can I do if my nuclear extract sample is too dilute?	In the EMSA User Manual section "Forming TF-DNA Complexes," the volume of nuclease-free water added to the mixture may be reduced so that a higher volume of nuclear extract can be added to achieve the recommended amount of nuclear extract for the protocol.
What is the cold probe and what is it used for?	The cold probe is the unlabeled (not biotinylated) version of the probe. The cold probe is used as a competition assay to help verify that the shifted band is your transcription factor (TF) of interest. A cold probe version is supplied with each EMSA Kit or EMSA Probe order.

<p>What is the EMSA Combo Kit (Catalog No. AY999) ?</p>	<p>The EMSA Combo Kit (Catalog No. AY9999) allows you to order any three EMSA probes. You will receive the standard tube of labelled probe (25 rxns) and cold probe (25 rxns) for each of the three probes. In addition, you will also receive the EMSA Kit - sufficient for running five 8 x 10 cm gels. When ordering AY9999, please specify the catalog numbers for the three probes by placing a "P" at the end of each catalog number. For example, for the NFkB (1) probe, use AY1030P.</p>
<p>What is the pH for 10X TBE stock solution?</p>	<p>The pH of the TBE is 8.4 +/- 0.1. We use the 10X TBE stock solution from Invitrogen (catalog# 15581-044).</p>
<p>What mJ setting should be used to UV crosslink membrane for EMSA?</p>	<p>We use the AutoCross linking feature on the Stratagene Stratalinker UV Crosslinker 2400. The AutoCross linking feature uses a total of 120 mJ to crosslink the membrane.</p>
<p>Where can I stop in the EMSA protocol?</p>	<p>You can stop after UV crosslinking the membrane after transfer and leave the blot out to dry at room temperature (Step 2 of Immobilization and Detection). The membrane is stable for several months if stored between Whatman paper and in the dark.</p>
<p>Why isn't the cold probe is not making my shifted band fainter?</p>	<p>The cold (competitive)probe has higher concentration than the label probe so it should at least make the band look fainter. Please ensure that you add the cold probe to the TF-DNA complex mix first before the addition of the labelled probe. When the cold, competitive probe successfully out-competes the labelled probe, the shifted band will appear fainter or disappear altogether.</p>