Gel shift protocol for the LightShift® Chemiluminescent EMSA Kit

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Anneal oligos

1. Spin down the lyophilized oligos and resuspend in TE buffer at 100 pmol/µl.
2. Dilute each oligo to 1 pmol/µl in TE.
3. Mix 1 µl of each forward and reverse oligo in 48 µl of TE to a final concentration of 20 fmol/µl.
4. Incubate the oligos at 95°C for 5 min. Switch off the heat block and let it cool down to room temperature (that will take 2-3 hours).
5. Keep oligos on ice and store at -20°C.

Prepare and Pre-run Gel

A native polyacrylamide gel will be needed. The appropriate polyacrylamide percent depends on the size of the target DNA and the binding proteins. It is recommended to use a 6% gel.

5X TBE pH 8.3
450 mM Tris
450 mM boric acid
10 mM EDTA

6% non-denaturing neutral polyacrylamide gel in 0.5X TBE
One 8x8x0.1 cm gel (pour without stacking gel):
600 µl 5X TBE [0.5X final]
1.2 ml 30% polyacrylamide solution [6% final]
60 µl 50% glycerol [final 0.25%]
4 ml MilliQ
10 µl TEMED
40 µl 10%APS

1. Pour gel, insert comb, let polymerize.
2. Fill electrophoresis unit with 0.5X TBE. Pre-run gel for 30-60 min at 100 Volts.

Binding reaction

1. Thaw all binding reaction components and place on ice. Avoid excessive warming!
2. Set up binding reactions. For a minimal binding reaction of 20 µl:
   - x µl Milli Q
   - 2 µl 10X binding buffer (1X final)
   - 1 µl 1µg/µl poly dI•dC (50 ng/µl final)
   - x µl unlabeled oligo (4 pmol final) Add ONLY in competitive rxn
   - x µl* nuclear extract/protein/MQ
   - x µl BSA (3 µg/µl final) add when purified protein is being used
   - ADD Labeled DNA LAST!! 1 µl labeled oligo (20 fmol final)
3. Incubate the binding reaction at room temperature for 20 min.

*In the literature, proposed amounts of proteins and oligos used for EMSAs are very different. We have seen different groups use: from 5 µg protein and 2 nM, 10 µg protein and 0.5 ng oligo or 4 µg nuclear extract and 20 fmol oligo.

**Additionally, Glycerol, NP-40, KCl, $MgCl_2$ and/or EDTA can be added to the binding reaction, please see Kit's Instructions for a starting point.

***Since I was using the same protein with different oligos, I made a master mix of everything except the oligos, and incubated it on ice for 2-3 min. Meanwhile, I distributed the oligos into respective microcentrifuge tubes, added the master mix afterwards, and mixed by pipetting gently up and down.

Run gel on Binding Reactions

1. Flush the wells of your gel.
2. Add 5 µl of 5X Loading Buffer to the binding reaction. Gently pipette up and down to mix. Do not vortex or mix vigorously!
3. Load 20 µl of each reaction.
4. Run gel at 100 V until the dye has migrated ¾ down the length of the gel. Running time depends on the oligo size. The lowest dye runs at about 50 bp.

Transfer to membrane

1. Cut positive charged nylon membrane 8x10 cm.
2. Soak membrane in 0.5X TBE for at least 10 min.
3. Make sure to cut the gel and the membrane to ensure right orientation.
4. Assemble blot per manufacture's instruction.
5. Transfer in 4ºC room in pre-cooled water O/N 10V, 260 mA or for 2h 25V, 300 mA.
6. Disassemble blot.
7. Place the membrane with the BMB side up on a dry paper towel. There should be no dye remaining in the gel. Let liquid soak into membrane, but do not let the membrane dry out! Immediately crosslink.

Crosslink

1. Place membrane face down on a transilluminator (covered with plastic wrap).
2. Incubated for 15 min with the 302 nm setting.
3. The membrane may be stored dry at room temperature for several days, but do not allow the membrane to get wet again until ready to proceed with developing.

Develop blot

Perform all blocking and detection incubations in clean trays on an orbital shaker.

1. Briefly warm the Blocking buffer and 4X Washing Buffer to 37-50ºC in a water bath until all particles dissolve.
2. Incubate membrane in 20 ml Blocking Buffer for 15 min.
3. Mix 20 ml blocking buffer with 66.7 µl Stabilized Streptavidin-hrp conjugate.
4. Incubate membrane 15 min in this solution.
5. Dilute 40 ml of 4X Washing Buffer with Milli Q to make 120 ml of washing solution.

6. Change container. Rinse briefly with 20 ml of washing solution. Wash 4X with 20ml for 5 min.
7. Change container. Incubate with 30 ml of Equilibration Buffer for 5 min.
8. Prepare Substrate Working Solution by adding 6 ml Luminol/Enhancer Solution to 6 ml Stable Peroxide Solution (protect from light).
9. Remove membrane from Equilibration Buffer, gently blot edge on a paper towel to remove excess buffer, and place in a clean tray.
10. Pour Substrate Working Solution on membrane and incubate for 5 min (in the dark). Blot membrane edge on paper towel, wrap in plastic wrap and expose X-ray film.