Filter Binding Assay for EBNA-1

Last Modified 5.2.2003 by Scott E. Lindner

1. Wash one 0.45um Millipore nitrocellulose filter (Cat# HAWP01300) in 1ml 0.4M KOH for 1 min; wash in 3ml dH_2O for >1min.
2. Assemble filter apparatus: white plastic bottom support/funnel, nitrocellulose filter, white plastic top with syringe adapter, 3ml syringe
3. Pre-incubate filter with 100ul Binding Buffer at filter binding temperature (4C) until ready to bind DNA/EBNA-1 solution (e.g. conduct in 7^{th} floor cold room).
4. Incubate DNA fragment of interest with DNA with no known binding sites in a total volume of 100-250ul Binding Buffer.
5. Incubate 4ug 6xHis-dnEBNA-1 in total volume of 100ul Binding Buffer.
6. Pre-incubate both the DNA and Protein solutions at 37C for 5min.
7. Mix the DNA and Protein solutions by pipetting, incubate for 15min, 37C. Shift solution to binding temperature by addition of ice cold Binding Buffer to a final volume of 1000ul, incubation on ice until loading on filter
8. Pass pre-incubation through filter by increased pressure supplied by syringe above filter. Remove syringe from filter apparatus.
9. Load DNA/EBNA-1 solution in upper portion of the filter apparatus, reattach syringe above. Filter all of solution through at slow rate (1ml/1min). Remove syringe and withdraw plunger from syringe. Reattach syringe. Reload flowthrough into filter apparatus and incubate at filter binding temperature (4C) for 10min. Pass through filter as above and collect in a labeled tube.
10. Load 400ul Wash Buffer into the syringe. Reattach the plunger and wash the filter immediately, collecting fraction in a separate tube. Remove syringe and withdraw plunger from syringe. Reattach syringe.
11. Repeat wash as per step 10, but pass through the filter when you are immediately ready to add elution buffer
12. Shift membrane to elution temperature (RT). Remove syringe, load 100ul elution buffer to top chamber of filter apparatus. Reattach syringe and allow the solution to incubate for 10min at elution temperature (RT). Pass through filter, repeat with a second 100ul elution.
13. Add 1/10 volume 3M Sodium Acetate, 1ul glycogen (20ug/ul stock), and 2-3 volumes 100% EtOH, mix by inversion
14. Spin 30min, 4C. Decant supernatant, invert on sterile Kimwipe.
15. Spin 5min, 4C. Pipette residual supernatant, allow to partially dry. Resuspend in 20ul 1xTE.
16. Load on 0.8% TAE agarose gel, visualize by EtBr staining by UV.
17. Quantify percent of each band present by comparison to input DNA fragment used.

(Optional) Quantify protein in each fraction by SDS-PAGE/Western.

Binding Buffer:
10mM HEPES
10mM $MgCl_2$
150mM NaCl
Add 5% DMSO fresh

Wash Buffer:
10mM HEPES
10mM $MgCl_2$
150mM NaCl
10% glycerol
Add 5% DMSO fresh

Elution Buffer:
10mM HEPES
150mM NaCl
0.2% SDS