DIG High Prime DNA Labeling and Detection Kit Protocols

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Standard DNA Labeling Protocol

1. Add 1 ug (10 ng - 3 ug) template DNA and sterile water to a final volume of 16 ul to a reaction vial.
2. Denature the DNA by heating in a boiling water bath for 10 min. Quickly chill in an ice/NaCl or ice/ethanol bath.
3. Add 4 ul DIG-High Prime, mix and centrifuge briefly.
4. Incubate for 1-20 hr at 37 C.
5. Stop the reaction by adding 2 ul 0.2 M EDTA and/or heating to 65 C for 10 min.

Quantification of labeling efficiency

1. Dilute an aliquot of DIG-labeled DNA to 300, 100, 30, 10, and 3 pg/ul
   - Dilute to approx. 1 ug/ml (1ng/ul, 1000pg/ul) =A
   - Dilute A 1:3.3 -- 10 ul in 23 ul H2O =B (300 pg/ul)
   - Dilute A 1:10 -- 5 ul in 45 ul H2O =C (100 pg/ul)
   - Dilute B 1:10 -- 5 ul in 45 ul H2O =D (30 pg/ul)
   - Dilute C 1:10 -- 5 ul in 45 ul H2O =E (10 pg/ul)
   - Dilute D 1:10 -- 5 ul in 45 ul H2O =F (3 pg/ul)
2. Apply a 1 ul spot of each of the 5 dilutions onto the marked squares of a quantification test strip. Air dry for approx. 2 min. (Write only on the polyester carrier area.)
3. Dip the teststrips (one quantification and one control back to back) in 2 ml of the following solutions for the appropriate times. Between steps, let excessive liquid drip onto a tissue paper.

vial 1	buffer 2 - blocking	2 min
vial 2	antibody solution	3 min
vial 1	buffer 2 - blocking	1 min
vial 3	buffer 1 - washing	1 min
vial 4	buffer 3 - equilibration	1 min
vial 5	color substrate (DARK)	5-30 min

4. Stop the color reaction after a max of 30 min. by briefly rinsing in water. Air dry on 3MM Whatman paper, protected from light.
5. Determine quantity of DIG labeled DNA by comparing color intensity with control strips.

SOLUTIONS (DIG Wash and Block Buffer Set contains buffers 1-3)

Buffer 1: maleic acid buffer

0.1 M maleic acid, 0.15 M NaCl; adjust to pH 7.5 with solid NaOH

Buffer 2: blocking solution

Dilute 10X solution in vial 6 1:10 in maleic acid buffer

Buffer 3: detection buffer

0.1 M Tris, 0.1 M NaCl, 50 mM MgCl2, pH9.5

Antibody solution

Dilute vial 4 (anti-dioxigenin-AP) 1:2000 in buffer 2

Color substrate solution
Freshly prepared: 40 ul of NBT/BCIP stock to 2 ml buffer 3
Hybridization protocol for Southern blots
1. Prewarm appropriate volume of hybridization buffer (20 ml/100 cm2) to hybridization temperature and incubate filter for 30 min with gentle agitation.
2. Denature DIG-labeled DNA probe (5-25ng/ml) by boiling for 5 min and rapidly cooling on ice water.
3. Add probe to prewarmed hybridization buffer (2.5ml/100 cm2 membrane) and mix well but avoid foaming.
4. Pour off prehybridization solution and add probe mixture.
5. Incubate with gentle agitation for at least 6 hrs. Probably best overnight - 16 hrs.
6. Wash 2 x 5 min in ample 2X SSC, 0.1% SDS at room temp.
7. Wash 2 x 15 min in 0.5 SSC, 0.1% SDS at 68 C under constant agitation

SOLUTIONS
10x SSC: 0.15 M NaCitrat, 1.5 M NaCl, pH 7.0
10% SDS
DIG Easy Hyb **or**
Standard Hybridization Buffer
- 5X SSC
- 0.1 % (w/v) N-lauroylsarcosine
- 0.02 % (w/v) SDS
- 1% Blocking reagent (1/10 volume of 10x blocking solution)

Detection
1. Rinse membrane 1-5 min in washing buffer.
2. Incubate for 30 min in 100 ml blocking solution/buffer 2
3. Dilute anti-DIG-AP conjugate to 75 mU/ml (1:10000) in buffer 2.
4. Incubate membrane for 30 min. in 20 ml antibody solution.
5. Wash 2 x 15 min in 100 ml washing buffer.
6. Equilibrate 2-5 min in 20 ml detection buffer/buffer 3.
7. Place membrane (DNA side up) on a development folder and apply approx. 20 drops (1 ml) CSPD solution out of the dropper bottle. IMMEDIATELY cover the membrane with the second sheet of the folder to spread the substrate evenly and without air bubbles over the membrane. Incubate 5 min.
8. Squeeze out excess liquid and seal the edges of the development folder.
9. Incubate the damp membrane for 5-15 min at 37 C.
10. Expose to X-ray film for 15-25 min at room temp.

SOLUTIONS (DIG Wash and Block Buffer Set contains buffers 1-3)
Buffer 1: maleic acid buffer
0.1 M maleic acid, 0.15 M NaCl; adjust to pH 7.5 with solid NaOH

Washing buffer
Maleic acid buffer plus 0.3% (w/v) Tween 20

Buffer 2: blocking solution
Dilute 10X solution in vial 6 1:10 in maleic acid buffer

Buffer 3: detection buffer

0.1 M Tris, 0.1 M NaCl, pH9.5