TechTip 125
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# Gold Hybridization Buffer for use in ECL Direct Labeling System

On-going development work at Amersham Biosciences has led to an improved hybridization buffer for use with the non-radioactive Enhanced Chemiluminescent Direct labeling (ECL) system (RPN.3000). The new ("Gold") buffer supplied with this system employs a novel rate enhancer in 6M Urea for use with DNA or RNA probes directly labeled with Horseradish Peroxidase (1,2). Standard ECL signal development is used to generate high quality results on Hyperfilm ECL. The Gold buffer offers the following advantages over the previous buffer in the ECL Gene Detection System:

A. Higher sensitivity
B. Lower backgrounds
C. No crystallized components
D. Longer ECL light emission
E. Lower probe concentration with nitrocellulose blots
F. Equivalent sensitivities with both nylon and nitrocellulose

The following Northern blot comparison was done comparing Gold Buffer with the previous ECL Gene Detection buffer:

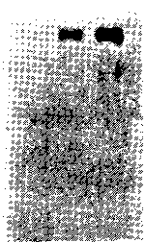
| | Gold Buffer | Standard ECL Buffer |
|---|---|---|
| Amount Loaded | 0.05 0.5 1.0 µg | 0.05 0.5 1 .0 µg |
| 2.6 Kb → |  |  |

Figure 1. Duplicate samples of Poly (A) + RNA from heat shocked (42° C - 60 minutes) HeLa cells were run on a MOPS/0.66M Formaldehyde agarose gel (3) and capillary blotted to Hybond ECL. Blots were baked for 2 hours at +80° C. Hybridization was overnight at +42° C with 0.25 ml buffer/c& membrane. Probe concentration was 10 ng/ml for Gold buffer and 20 ng/ml* in previous ECL hybridization buffer. Both buffers contained 0.5M NaCl. Probe was the cloned insert from pH-HSP70, containing the coding sequence for the human HSP70 gene (4). Post-hybridization washes were in ECL primary wash buffer minus Urea - 2 X 20 minutes at +55° C (5). 60 minute exposure to Hyperfilm ECL.

*Previous ECL Gene Detection system used 20 ng/ml for nitrocellulose blots

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## Other Important Features of ECL Gold Buffer

1. Blocking agent (supplied) must be added to a final concentration of 5% (w/v) for both nitrocellulose and nylon membranes prior to use. Slowly add the required amount of block to buffer stirring on a magnetic stirrer, stir for 1 hour. AVOID LARGE CLUMPS of blocking reagent. Preheat buffer to 42°C for 0.5 to 1 hour.

2. Buffer is supplied without NaCl. This is to allow the researcher the flexibility of altering the hybridization stringency based on homology of the probe/target. A 0.5M NaCl concentration will be sufficient for most applications and is the suggested starting point. Add NaCl to Gold buffer once blocking reagent is dissolved.

3. Use 10 ng/ml probe concentrations with nylon and nitrocellulose blots.

## References


1. Renz, M. and Kurz, *C.,* (1884) *Nucleic Acids Research.* Vol. 12, pp. 3435-3444

2. Durrant, I., Benge, L., Sturrock. C., Devenish A., Howe, R., Roe, S.. Moore, M., Scouafava G., Proudfoot, L., Richardson, T., and McFarthing, K., (1990) *Biotechniques,* Vol. 8, No. 5, pp.564-570.

3. Davis, L.G.. Dibner, M.D., Battey, J.F., (1986) *Basic Methods in Molecular Biology,* Elsevier Science Publishing Co. Inc.., New York.

4. Wu, B., Hunt, C., Morimoto,R., (1885) *Molecular and Cellular Biology,* Vol. 5 No. 2, pp. 330-341

5. The ECL Gene Detection System Technical Manual, Amersham International. plc.