



Stability of Biotinylated DNA Standard

Purpose:

To evaluate the stability of the Biotinylated DNA Standard, a component of the DNA Biotinylation Kit (Cat. No. 60-01-00).

Reagents:

Samples of the biotinylated DNA standard pilot lot were stored at -20°C, 4°C and room temperature for 22 weeks (150 days). Reference samples were stored at -20°C and freshly thawed on the date of each assay. Sample performance was evaluated biweekly by dot ELISA.

Test Parameters:

Samples were evaluated by dot ELISA test procedure on nylon membrane (S&S Nytran) as follows:

1. Prepare two-fold dilutions of biotinylated DNA standard from 1 µg/ml stock: 500 ng/ml; 250 ng/ml; 125 ng/ml; and 62.5 ng/ml.
2. Apply 1.0 µl of each dilution to the appropriate spot on the membrane strips (Figure 1) using a microdispenser. UV-crosslink DNA to the membrane and air dry 10 minutes.
3. Block strips with Milk Diluent/Blocking Buffer (Prod. Code 50-83-00), Lot RH75, diluted 1:4 in reagent quality water, one hour at room temperature.
4. Incubate strips with Peroxidase-Labeled Streptavidin (Catalog No. 474-3000), Lot RM22, diluted 1:1,000 in diluted blocking solution, 30 minutes at room temperature.
5. Wash strips 3 times for 3 minutes each using Wash Solution Concentrate (Prod. Code 50-63-02), Lot QJ45, diluted 1:20 in reagent quality water.
6. Develop strips in TMB Membrane Substrate (Prod. Code 50-77-02), Lot PL10, 5 minutes at room temperature.
7. Stop substrate reaction after 5 minutes by rinsing the membranes in reagent quality water 30 seconds.
8. Allow strips to air dry and store sealed under plastic in the dark.

Results:

All samples developed with equal intensity in color to an endpoint of 62.5 ng/ml. The room temperature sample showed a slight loss of sensitivity after 14 weeks storage (see Fig. 1).

Conclusions:

KPL's Biotinylated DNA Standard demonstrates excellent stability when stored at -20°C or 4°C for 150 days, and is expected to remain stable for at least one year from the date of manufacture when stored at -20°C.

Figure 1.

Weeks 1 - 12

dilutions from 1 µg/ml - 62.5 ng/ml

Ref.	●	●	●	●	○															
-20°C	●	●	●	●	○															
4°C	●	●	●	●	○															
RT	●	●	●	●	○															

Weeks 14 - 22

dilutions from 1 µg/ml - 62.5 ng/ml

Ref.	●	●	●	●	○															
-20°C	●	●	●	●	○															
4°C	●	●	●	●	○															
RT	●	●	●	●	○															