Invitrogen™
life technologies

Instruction Manual

DNA DipStick™ Kit

For quantitation of nucleic acids

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General Information

Introduction

The DNA DipStick™ Kit is ideal for estimating the concentration of single- or double-stranded DNA, RNA, or oligonucleotides (of 6 bases or more) at concentrations as low as 0.1 ng/µl. The DNA DipStick™ Kit provides an accurate measurement of nucleic acids for critical procedures such as PolyA$^+$ RNA isolation, cDNA library construction, DNA subcloning, PCR and RT-PCR, RFLP analysis, DNA sequencing, nucleic acid elution, and RNA transcription.

Permanent visual results are produced within 10-15 minutes without the need for photography. The color developed from a sample's spot on a DNA DipStick™ will remain indefinitely and can be pasted directly into a notebook for future reference.

Contents and Storage

The components included in the DNA DipStick™ Kit are listed below. Sufficient reagents are provided in the kit to perform 50 nucleic acid quantitation assays.

Store the kit at room temperature. All reagents are guaranteed stable at room temperature for 6 months when stored properly.

Item	Amount
DNA DipSticks™	50
Coupling Solution	60 ml
Developer	4 ml
Developer Stock	60 ml
Reaction Cuvettes	6
Wash Solution	60 ml
Standard DNA	100 ng
Quick Reference Card (with [DNA] standards)	1

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General Information, Continued

Safety

The Coupling Solution contains cacodylic acid and the Developer contains potassium ferrocyanide. Both are considered hazardous substances. Use gloves, a lab coat, and protective eye wear when handling these solutions. Dispose of the Coupling and the Developing Solutions according to local waste disposal regulations.

Product Qualification

The sensitivity of the detection of nucleic acids with the DNA DipStick™ is verified using Standard DNA included in the kit in a quantitation assay as described in this manual. The assay must detect the full range of standards.

Accurate results are verified by visual examination of the color intensities of the dilutions and their comparison with the included Concentration Standard Chart.

Using the DNA DipStick™ Kit

Introduction

Instructions for using the DNA DipStick™ are provided below.

Supplied by the User

- Deionized water in a squirt bottle
- Sterile TE or water

Starting Material

Nucleic acid preparations used for quantitation with the DNA DipStick™ should be pure and solubilized in TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA) or deionized/distilled water. Certain solutions or chemicals may interfere with the accuracy of the result (see page xx).

Avoid using carriers such as herring sperm DNA or tRNA for alcohol precipitation of DNA samples to be analyzed by the DNA DipStick™. You can use glycogen and linear acrylamide as carriers for precipitation.

Note

The DNA DipStick™ assay provides linear results from 0.1 to 10 ng of nucleic acid. To obtain accurate readings it is important that the sample concentration fall within this range. We recommend applying dilutions of the sample to the same DNA DipStick™ (typically 1:10 and 1:100 dilutions in sterile water or TE are sufficient).

Before Starting

Six cuvettes are supplied with the kit to perform two DNA DipStick™ assays simultaneously, if desired. Using a water-proof, permanent marker label the uncapped cuvettes as described in the table below. This ensures that each cuvette is used with the same solution during subsequent uses of the DNA DipStick™ Kit and minimizes the chance of cross contamination.

Cuvette	Label As	Solution
1 and 2	#1	Wash Solution
3 and 4	#2	Coupling Solution
5 and 6	#3	Developing Solution (Developer Stock + Developer)

Continued on next page

Using the DNA DipStick™ Kit, Continued

Control DNA

The control DNA is included in the DNA DipStick™ Kit to enable you to quantitate nucleic acid in your sample. **The control DNA tube is labeled as "Standard DNA"** and contains 100 ng of lyophilized supercoiled plasmid DNA.

To use the control DNA, follow the instructions below:

1. Prepare a 10 ng/µl solution of the control DNA by resuspending the control DNA in 10 µl of sterile water.

   Note: Store the control DNA at -20°C after reconstitution.

2. Dilute the 10 ng/µl solution of the control DNA to 1:10 and 1:100 to obtain three concentrations (10 ng/µl, 1 ng/µl, 0.1 ng/µl).

3. Spot 1 µl of each of the three concentrations on the DNA DipStick™ and develop according to the protocol on pages 5-8. You may use this control DNA DipStick™ in place of the standards shown on the Quick Reference Card.

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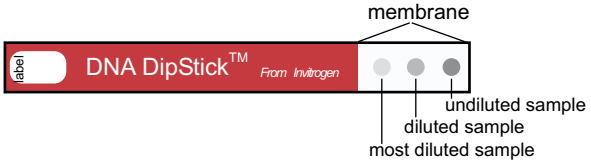
Using the DNA DipStick™ Kit, Continued

Applying Sample

1. Make appropriate dilutions of the sample to be tested in sterile water or TE (bring the final concentration between 0.1 and 10 ng/µl). For unknown sample concentrations, try the undiluted sample along with dilutions of 1:10 and 1:100 (see figure below)



2. If you are using the control DNA included in the kit, prepare dilutions of the control DNA as described on the previous page.
3. Avoiding contact with the membrane portion of the stick, place one DNA DipStick™ per sample (membrane up) on a clean surface (the sample and its two dilutions can all be applied to the same DNA DipStick™).
4. Apply 1 µl each of the sample directly on to the membrane without overlapping the spots.
5. Allow the spots to air dry for 5-10 minutes. You can place the DNA DipStick™ under a light source for a few minutes to speed up the drying process.

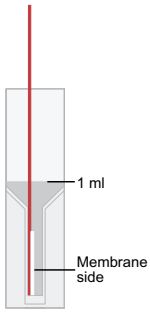
   Note: Consistency is important when applying spots. Avoid contact between the membrane and the pipette tip. The sample may remain as a tight drop for a few seconds after application and then absorb into the membrane, forming an irregularly shaped spot. This will not affect the result of the assay in any way. If adjacent samples fuse together at this stage, the final result will be a round, blue-colored dot.
6. While the sample spots are drying, set up the solutions to develop the assay. To open the Developer, press upward firmly on the red cap.

Continued on next page

Using the DNA DipStick™ Kit, Continued

Procedure

1. To the labeled cuvettes from page 3, add
   - 1 ml of Wash Solution in Cuvette #1
   - 1 ml of Coupling Solution in Cuvette #2
   - 1 ml of Developer Stock plus 1 drop of Developer into Cuvette #3. Cap Cuvette #3 and mix the Developing Solution by inverting.
2. Once the sample spots have dried, place the DNA DipStick™ in Cuvette #1 (containing Wash Solution) for 10 seconds.
3. Transfer the DNA DipStick™ into Cuvette #2 (containing Coupling Solution) and let it stand for 3 minutes. Place the DNA DipStick™ vertically into the solution with the back of the DNA DipStick™ against the cuvette wall to allow complete contact between the membrane and the Coupling Solution (see figure below).



4. Remove the DNA DipStick™ and rinse it with deionized water for 20 seconds, and place it back into the Wash Solution in Cuvette #1 for 4 minutes. The DNA DipStick™ must be vertical with the back of the stick against the cuvette wall.
5. Place the DNA DipStick™ into Cuvette #3 (containing the Developing Solution) for 2 minutes. The DNA DipStick™ must be placed as vertically as possible (see figure above).

Continued on next page

Using the DNA Dipstick™ Kit, Continued

Procedure, continued

6. Gently rinse the DNA DipStick™ in the Wash Solution in Cuvette #1. After 20 seconds remove the DNA DipStick™. Place it flat (with membrane side up) to air dry.
7. After drying the DNA DipStick™, compare the color intensities of the sample spots on the membrane to the control DNA or the standard chart on the DNA DipStick™ Quick Reference Card to estimate the concentrations of the samples.
8. Dispose of the Coupling Solution in Cuvette #2 and the Developing Solution in Cuvette #3 in accordance with local and state waste disposal regulations. Rinse all three cuvettes with distilled water and re-use the cuvettes for future assays.

Note

- The dots on the standard chart in the Quick Reference Card range in concentration from 0.1 ng/µl to 10 ng/µl and are applicable to single- and double-stranded DNA, RNA, and oligonucleotides.
- When comparing the sample dots to the standards, at least one of the sample concentrations should fall within the range of the standards,
- If the sample dots will are of intermediate intensity to those on the standard chart, then estimate a concentration value, or to achieve an optimal color match, prepare additional dilutions based on the initial result and repeat the assay.
- If the amount of the sample applied to the DNA DipStick™ ranges from 10-500 ng/µl, the intensity of the dot will not correlate to the amount of nucleic acid in the sample.
- If more than 500 ng/µl of nucleic acid is applied, the spot on the membrane will be white instead of blue.

Substances Interfering with the DNA DipStick™ Assay

Introduction

Common contaminants of DNA preparations interfering with the DNA DipStick™ assay are indicated below.

Reagent	Effect on the DNA DipStick™	
	Does not Interfere	Does Interfere
1X Ligation Buffer	•	
1X PCR Buffer	•	
Ammonium acetate < 0.75 M	•	
BSA < 10 ng/ml	•	
Crude preparation of phage DNA	•	
Deoxynucleotides <6 mM	•	
Low-melt agarose and Gelzyme™	•	
Glycogen < 0.1 mg/ml	•	
Linear acrylamide	•	
Molecular biology grade agarose		•
Polyacrylamide	•	
Polyethylene glycol	•	
Phosphate Buffer < 10 mM	•	
Sodium dodecyl sulfate (SDS)		•
Herring sperm DNA		•
tRNA		•

Technical Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

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Technical Service, Continued

MSDS Requests

To request an MSDS, visit our Web site (www.invitrogen.com).

1. On the home page, go to the left-hand column under 'Technical Resources' and select 'MSDS Requests'.
2. Follow instructions on the page and fill out all the required fields.
3. To request additional MSDSs, click the 'Add Another' button.
4. All requests will be faxed unless another method is selected.
5. When you are finished entering information, click the 'Submit' button. Your MSDS will be sent within 24 hours.

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