Product No.: 90-1024-01
Effective Date: 21Sep2012
Date Revised: 02 Jul 2012
Version: 01

ANP Technologies Inc.

NIDS® DIY Digoxigenin-NHS Antibody Labeling Kit

Part no. 90-1024-01

For Research Use Only

This procedure must be read in its entirety before using this product

Intended use

The purpose of the kit is to provide a rapid way to efficiently conjugate digoxigenin to an antibody.

Introduction

The ANP NIDS® DIY Digoxigenin-NHS Antibody Labeling Kit provides an easy way to label antibodies rapidly and efficiently. A simple, quality control check is included to verify that the conjugation is successful. The QC method uses less antibody reagent compared to the common HABA QC method. For best performance of the digoxigenin labeling reagents, please pair them with our NIDS® Streptavidin plates.

Materials Supplied by ANP Technologies, Inc:

- 10 mM Digoxigenin Reagent, Digoxigenin-NHS in DMSO: ANP, Part no. 90-1025-50, 50 µL. **Store at -70°C.**
- Labeling Buffer: ANP, Part no. 90-1020-00, 20 mL. Store at 4°C.
- Digoxigenin Labeling QC Strip: ANP, Part no. 90-1027-00, 2 strips. Store at 2-8°C.
- QC Buffer: ANP, Part no. 90-1029-00. 1 mL. Store at 4°C.
- 30 kDa Centrifugal filter unit: Millipore, Cat. No. UFC803096

Other Necessary Materials/Equipments Not Supplied by ANP Technologies, Inc:

- Antibody
- 10 mM Phosphate Buffered Saline (PBS) pH 7.4
- Balance
- UV/Vis Spectrometer
- Vortex shaker
- Centrifuge
- Appropriate reaction vials
- Pipette and sterile pipette tips

General instructions and Limitations

- Remove 10 mM Digoxigenin Reagent from -70°C storage and allow it to equilibrate at room temperature before opening.
- The components of the kit should not be used beyond the expiration dates.
- Variations from the stated procedure may yield erroneous results.
- Avoid microbial contamination of reagents and buffers. This may affect assay results.

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- Individual results may vary due to differences in technique, reagent preparation, containers, etc.

Safety

It is important to **wear gloves when handling the 10 mM Digoxigenin Reagent**, because it is a toxic reagent. Handle all other materials with proper personal protective equipment.

Calculations

- Determine the mass of antibody to be digylated and calculate the volume of 10 mM Digoxigenin Reagent required to add to the antibody to maintain a recommended molar ratio of 15:1:

$$\frac{\text{mg Antibody}}{\text{MW}_{\text{antibody}}\ \text{mg/mmol}} \text{ x } \frac{15\ \text{mmol Digoxigenin Reagent}}{1\ \text{mmol Antibody}} \text{x } \frac{10^6\ \mu\text{L}}{10\ \text{mmol}} = \mu\text{L Digoxigenin Reagent required}$$

Example for 0.5 mg of an IgG type antibody (MW=150,000 mg/mmol):

$$\frac{0.5\ \text{mg IgG}}{150000\ \text{mg/mmol}} \text{x } \frac{15\ \text{mmol Digoxigenin Reagent}}{1\ \text{mmol IgG}} \text{ x } \frac{10^6 \mu\text{L}}{10\ \text{mmol}} = 5\ \mu\text{L Digoxigenin Reagent required}$$

Digoxigenin Labeling Procedure

1. Measure the absorbance of the antibody at 280 nm. For solids, dissolve the antibody in PBS first and then perform the measurement. Divide by the extinction coefficient to determine the concentration of the antibody.

2. Add 0.5-2 mg antibody and 2 mL Labeling Buffer to the filter unit and centrifuge (suggested centrifuge settings are 6000 RCF for 15 minutes at 4ºC).

3. Discard filtrate from the filter unit, add 2 mL of Labeling Buffer and centrifuge again under the same settings. Repeat once.

4. Transfer the retentate from the filter unit into an appropriately sized tube. Rinse the filter unit with ~200 µL Labeling Buffer and transfer the rinse solution into the tube; repeat once. Dilute the antibody to about 1 mg/mL using Labeling Buffer; this solution will be designated solution **A**.
   Note: Perform QC steps 1, 2, 4 and 5 using solution A.

5. Add the required volume of 10 mM Digoxigenin Reagent (calculated previously) to the tube containing solution **A** and mix well. Allow the reaction to incubate on a shaker on low speed for 1 hour at room temperature.

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6. Transfer the reaction mixture from the reaction tube to the previously used filter unit. Rinse the reaction tube with ~200 µL PBS and transfer the rinse solution into the filter unit; repeat once.

7. Add 2 mL of PBS to the filter unit and centrifuge (suggested centrifuge settings are 6000 RCF for 15 minutes at 4ºC).

8. Discard filtrate from the filter unit, add 2 mL of PBS and centrifuge again under the same settings. Repeat once.

9. Transfer the retentate from the filter unit into a vial with a screw cap. Rinse the filter unit with ~200 µL of the PBS and transfer the rinse solution into the vial containing the retentate; repeat once. Dilute the labeled antibody to about 1mg/mL with PBS; this solution will be designated solution **B**.
   Note: Perform QC steps 1, 3, 4 and 5 using solution B.

10. Measure the absorbance of **B** at 280 nm and divide by the extinction coefficient to determine the concentration of labeled antibody.

11. Store the labeled antibody using the same condition that is optimal for the non-labeled antibody.

QC test of Digoxigenin Labeled Antibody:

1. Remove two Digoxigenin Labeling QC Strips from their pouches.

2. Using a pipette, transfer a 0.1-0.5 µL drop of solution **A** onto the center area of the membrane of one strip. Designate this strip as the control strip. Wait 1 minute for the spot to dry.
   Note: For very small volumes allow capillary action to pull the drop onto the membrane surface; do not scratch the membrane of the strip.

3. Using a different pipette tip, transfer a 0.1-0.5 µL drop of solution **B** onto the center area of the membrane of the other strip. Designate this strip as the test strip. Wait 1 minute for the spot to dry.

4. Using an adjustable pipette, transfer 100 µL of the QC Buffer to the sample well of each of the strips. Allow to run for 10 minutes.

5. The control strip should develop no color. If the labeling was successful, the test strip will develop a red spot where the drop was placed; if the labeling was not successful, the spot will not develop a red color.

Disclaimer: If the control strip develops a red spot where the original antibody drop was placed, then it is recommended to use an ELISA method to QC the labeled antibody.

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Figure 1- Transferring a drop of solution onto the center area of the membrane of the QC Strip

Figure 2- Transferring QC buffer to the sample well of the QC strip

Figure 3- An example of a control strip with no color development, 0.5 µL of Unlabeled Mouse IgG was transferred onto the center area of the membrane of the QC Strip and then 100 µL of QC Buffer was transferred into the sample well of the QC Strip.

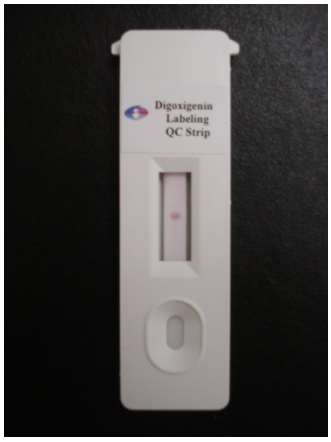

Figure 4- An example of a test strip with color development, 0.5 µL of Digoxigenin Labeled Mouse IgG was transferred onto the center area of the membrane of the QC Strip and then 100 µL of QC Buffer was transferred into the sample well of the QC Strip.

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