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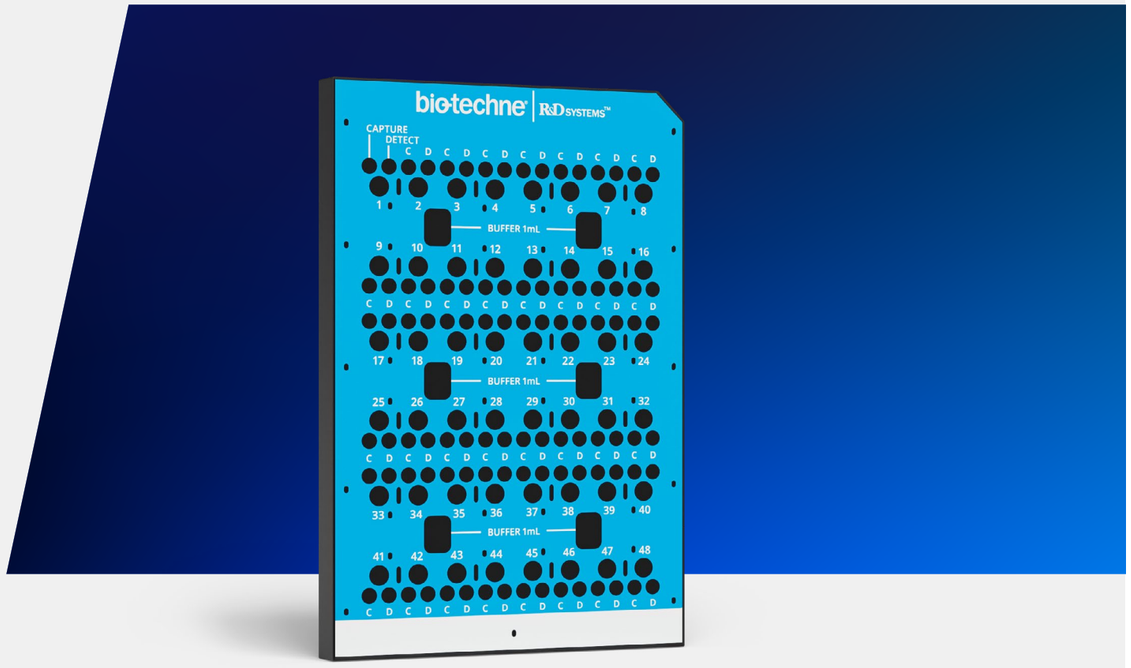
**Ella**
Custom Development Cartridge

## Quick Start Guide

# Simple Plex™ 48-Digoxigenin Cartridge




# Let’s get started

## Introduction

This guide will walk through the conjugation steps needed to prepare assay reagents for the 48-Digoxigenin Cartridge. The reagent conjugation is based on NHS-ester chemistry and is suitable for labeling molecules with amine groups.

The 48-Digoxigenin Cartridge can be used for a variety of assay configurations including antibody sandwich assays, antigen down assays, and assays using non-antibody affinity reagents.

For the purpose of this document, we will focus on the conjugation of antibodies for use in an assay.

Non-antibody affinity reagents containing amine groups may also be conjugated using the procedures outlined here.



## Reagents and Materials

### White box - store at 4°C

| Includes | Part Number |
|---|---|
| **48-Digoxigenin Cartridges (1)** | 952927 |
| **Wash Buffer (10 mL)** | 896055 |
| **5X Reagent Diluent (10 mL)** | 895182 |

### Other things you will need

| Reagents | Vendor | Part Number |
|---|---|---|
| **Digoxigenin Conjugation:** | | |
| Digoxigenin NHS ester | Enzo | ENZ-45022 |
| | Sigma-Aldrich | 55865 |
| N,N-Dimethylformamide (DMF) | Sigma-Aldrich | 270547 |
| **Biotin Conjugation:** | | |
| Biotin-XX, SE | Thermo Fisher | B1606 |
| | Sigma-Aldrich | B3295 |
| Dimethyl Sulfoxide (DMSO), ≥ 99% pure | Sigma-Aldrich | D2650 |
| **Required for both reactions** | | |
| Sodium Bicarbonate | Sigma-Aldrich | S8875 |
| **ELISA Plate-Coating Buffer – PBS** | R&D Systems | DY006 |
| Hinge-Cap Polypropylene Vials (1.7 mL) | Sigma-Aldrich | CLS3620 |
| UV-Star® Transparent Microplates (96-well, flat bottom) | Greiner Bio-One | 655801 |
| Zeba™ Spin Desalting Columns (40K MWCO, 0.5 mL) | Thermo Fisher | A57759 |
| Zeba™ Spin Desalting Columns (7K MWCO, 0.5 mL)*<br>*For use with proteins smaller than 40KD. | Thermo Fisher | 89882 |

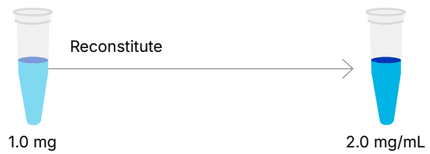
# 1. Reagent Preparation

## A
## Prepare Reagents

Proper PPE should be used throughout the protocol as digoxigenin is acutely toxic and DMF and DMSO are strong organic solvents.

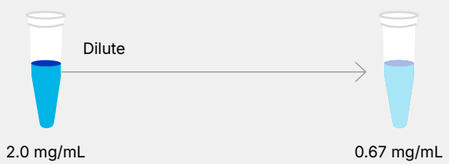
### Digoxigenin-NHS

1. Add 500 µL DMF
2. Vortex for 15-20 seconds
3. Solution can be stored at -20°C



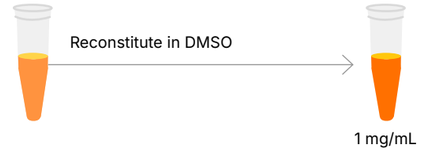

### Intermediate Digoxigenin-NHS

1. Add 20 µL of previously reconstituted Digoxigenin-NHS to 40 µL of DMF
2. Vortex for 15-20 seconds



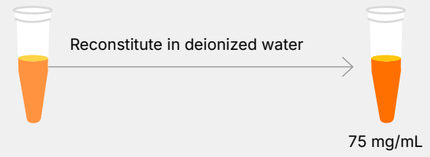

### Biotin-XX, SE

1. Weigh out biotin-XX and dilute with DMSO to make an 1.0 mg/mL solution
2. Vortex until solids are dissolved
3. Use within 15 minutes



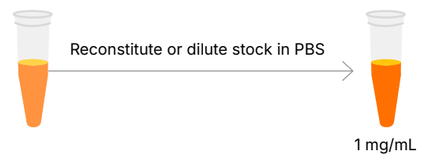

### Sodium Bicarbonate

1. Weigh out sodium bicarbonate and dilute in DI water to make a 75 mg/mL solution
2. Vortex until solids are dissolved




### Antibody

1. Dilute antibody stock to 1 mg/mL in PBS
2. Vortex each tube for 10 seconds and set aside

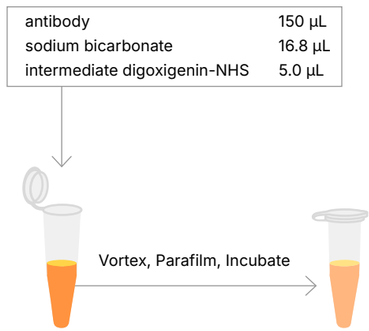



**NOTE:** If stock antibody solution contains carrier protein, remove by buffer exchange prior to conjugation.



## B
## Conjugate Digoxigenin-Antibody

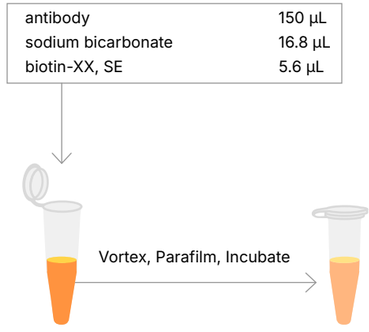
1. Combine the following:
   150 µL antibody working stock (1 mg/mL)
   16.8 µL sodium bicarbonate working stock (75 mg/mL)
   5.0 µL intermediate digoxigenin-NHS solution (0.67 mg/mL)
2. Vortex well and apply parafilm to the opening of each tube.
3. Incubate in the dark for 1 hour at room temperature.




**NOTE:** If using a protein other than an antibody as a capture reagent, this volume will have to be adjusted based on the 5:1 molar ratio of Dig-NHS to protein. Dig-NHS MW, 658.78 g/mole.

## C
## Conjugate Biotin-Antibody

1. Combine the following:
   150 µL antibody working stock (1 mg/mL)
   16.8 µL sodium bicarbonate working stock (75 mg/mL)
   5.6 µL biotin-XX, SE solution (1 mg/mL)
2. Vortex well and apply parafilm to the opening of each tube.
3. Incubate in the dark for 1 hour at room temperature.




**NOTE:** If using a protein other than an antibody as a detection reagent, this volume will have to be adjusted based on the 10:1 molar ratio of Biotin-NHS to protein. Biotin-NHS MW, 567.69 g/mole.

**NOTE:** For non-antibody affinity reagents, begin by conjugating with the molar excesses described (5 molar excess for digoxigenin, 10 molar excess for biotin). Some optimizations may be required. The formula below can be used to determine how much digoxigenin solution is required by accounting for the molecular weight of non-antibody reagents. The same basic formula can be used for biotinylation.

$$\left(\frac{\text{Protein mass}}{\text{Protein molecular weight}}\right) \times \text{molar excess of DIG} \times \left(\frac{\text{DIG molecular weight}}{\text{DIG solution conc.}}\right) = \text{mL of DIG solution needed}$$

**Using IgG as an example:**

$$\left(\frac{0.15\ mg}{150000\ g/mol}\right) \times 5\ molar\ excess \times \left(\frac{658\ g/mol}{0.67\ mg/mL}\right) = 0.005\ mL\ of\ DIG\ solution$$

## 2. Remove Free Label

### A
### Select the Appropriate Spin Column

1. For antibodies and other proteins larger than 40kD, use of 40K MWCO spin desalting columns is recommended.
2. For proteins with molecular weights between 7kD and 40kD, use of 7K MWCO spin desalting columns is recommended.

### B
### Remove Unreacted Label From Conjugates

1. Follow the spin column manufactures protocol to remove unreacted label.
2. When equilibrating the columns, PBS should be used.
3. Retain conjugates in 1.7 mL hinge cap vials and discard spin columns.



## 3. Quantitate Conjugates

### A
### Pipette Your Plate

1. Allocate 1 to 2 wells as blank and dispense 100 µL (0.100 mL) of PBS in each.
2. Dispense 100 µL (0.100 mL) of antibody-conjugate sample per well.
3. Read the microplate at 280 nm absorbance:
   - Enable blank subtraction
   - Record OD values for use in part B
4. Return each antibody-conjugate sample to its corresponding vial.

**NOTE:** If desired, protein concentration can be assessed by BCA assay immediately after elution from the spin column.
**Do no**t add 5X Reagent Diluent to samples prior to running BCA assay.

### B
### Calculate Initial Antibody Concentration

1. Divide each antibody-conjugate OD value by 1.4 to obtain the initial antibody concentration.
   - Record value for use in part E

**NOTE:** For non-antibody affinity reagents, the extinction coefficient will need to be determined to perform this calculation. 1.4 is specific to IgG molecules.

### C
### Calculate Pool Volume

1. Tare a scale using an empty 1.7 mL hinge-cap vial.
2. Weigh each antibody conjugate vial to obtain pool volume.
   - Record value for use in part D and E

**D**
**Calculate Reagent Dilutent Volume (optional)**

5X Reagent Diluent contains BSA which can be used as a carrier protein to stabilize the final reagent.

1. Use 5X Reagent Diluent concentrate, do not dilute.
2. For each antibody-conjugate sample in part C, divide the value obtained by 10.
3. Add the resultant mL of 5X Reagent Diluent to each sample prepared.
   · Record value for use in part E

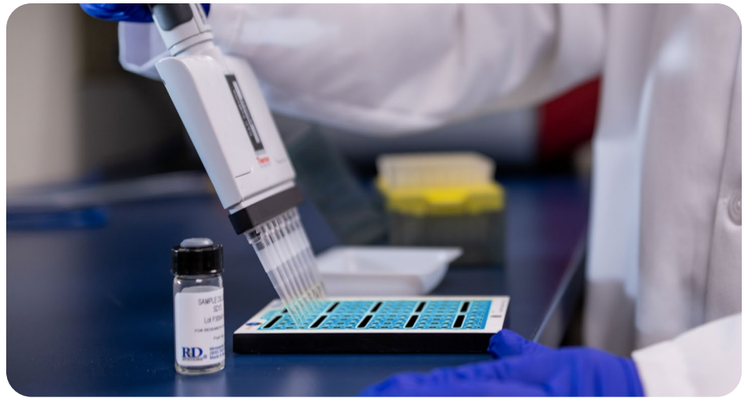
**E**
**Calculate Final Antibody Concentration**

1. Use the following formula:

$$\frac{\text{Initial Antibody Concentration (mg/mL)} \times \text{Pool Volume (mL)}}{\text{Pool Volume (mL)} + \text{Reagent Diluent Volume Added (mL)}} = \text{Final Antibody Concentration (mg/mL)}$$

2. Store samples at 4°C.

**NOTE:** Reagent stability will be dependent on the molecule conjugated.



## 4. Pipette Cartridge

For more consistent results, make sure the capture and detect reagents are sufficiently added and completely cover the bottom of the well.

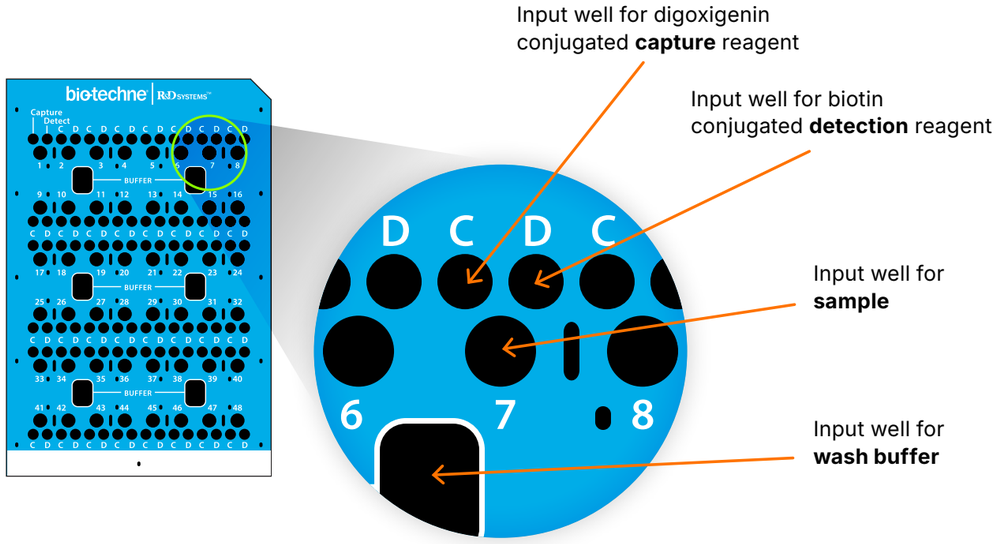
1. Prepare 25 mL of 1x Reagent Diluent

   Add 5 mL of reagent diluent concentrate to 20 mL of DI water.
   Mix thoroughly, avoid foaming.

2. Prepare working stocks of affinity reagents by diluting in 1x Reagent Diluent.

   A working concentration of 3.5 µg/mL is recommended for both capture and detection reagents. Further optimization may be required. Working stocks should be prepared fresh before each cartridge run.

| Well | Reagent | Volume | Input |
|---|---|---|---|
| **C** | Digoxigenin-Capture | 25-50 µL | 48 |
| **D** | Biotin-Detect | 25-50 µL | 48 |
| **1 - 48** | Sample | 50 µL | 48 |
| **Trough** | Wash Buffer | 1 mL | 6 |




3. Dispense capture and detection reagents into the cartridge. 50 µL is recommended to ensure complete coverage of the bottom of the well, but as little as 25 µL may be used.

   If the volume of capture and detect reagents is less than 50 µL ensure the liquid is properly settled by gently tapping the side of the cartridge to dislodge liquid that may be stuck to the side of the wells. DO NOT strike the bottom of the cartridge against a surface. This can cause liquid to spill and has the potential to damage the cartridge.

4. Dispense 50 µL of sample, standard, or control into each of their 48 wells.

   All wells, including capture and detection wells, must contain liquid. 1x Reagent Diluent can be dispensed into any unused wells.

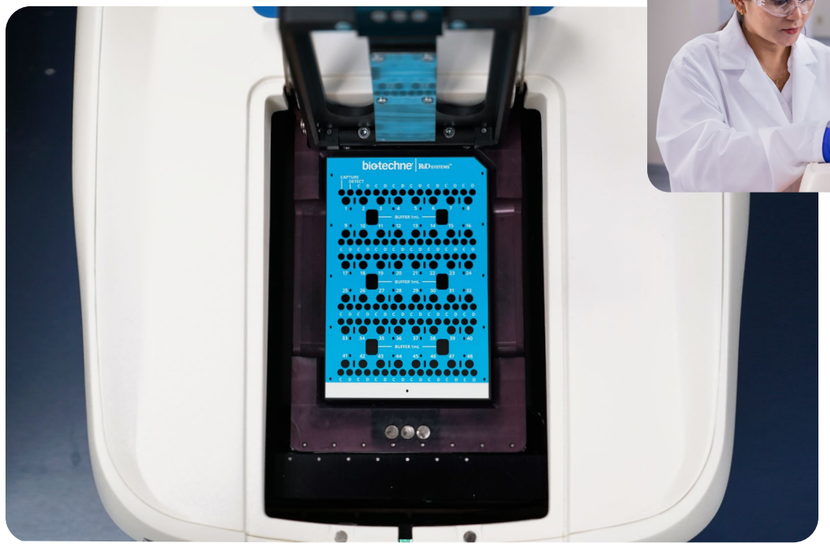
5. Add 1 mL wash buffer to each of the six wash buffer troughs.





## 5. Start Ella

1. Scan the kit barcode.
2. Scan the cartridge barcode for confirmation (if enabled).
3. Assign your samples to the cartridge inlets in Runner software.
4. Open Ella's door and lift the cartridge clamp.
5. Peel off the protective lining from the bottom of the cartridge.
6. Insert the cartridge into Ella's cartridge holder.
7. Close Ella's cartridge clamp and door.
8. Confirm your selections and click the Start button in Runner software.
9. When the run is complete, discard the cartridge.



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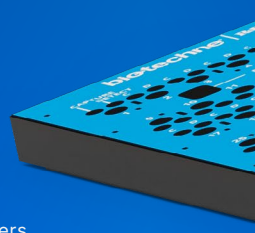



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