**Individual Run Sheet – Visualizing the Biotinylated Dextran Amine (BDA)**

Bird ID: \_\_\_\_\_\_\_\_\_\_\_\_\_\_

Reagent: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Bleach Solution (per tray, 18.7 mL)**

* *30% Hydrogen peroxide (H2O2) 700 μL*
* *Methanol (CH3OH) 9 mL*
* *1x PBS 9 mL*

**Streptavidin Solution (per tray, ~20 mL)**

* *0.4% PBS-Tx 20 mL*
* *Streptavidin (fluorescent)*

*or*

*Streptavidin – HRP (chromogenic) \_\_\_ μL*

1. Wash sections in 1x PBS for 3x5 minutes. ☐ ☐ ☐
2. Bleach sections for 20 minutes.

|  |  |  |  |
| --- | --- | --- | --- |
| **1 mL solution per section** | **Methanol** | **30% Hydrogen Peroxide** | **1x PBS** |
| 1 section |  |  |  |
| 20 sections | 9 mL | 700 µL | 9 mL |

1. Wash sections in 1x PBS for 5x5 minutes. ☐ ☐ ☐ ☐ ☐
2. Incubate sections in **reagent** (\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_) (dilution 1: \_\_\_\_) + 0.4% PBS-Tx for 1 hour at room temperature on the rotator.

|  |  |  |
| --- | --- | --- |
| **1 mL solution per section** | **Reagent** | **0.4% PBS-Tx** |
| 1 section | \_\_\_\_\_ µl | 1 mL |
| 20 sections | \_\_\_\_\_ µl | 20 mL |

1. Wash sections in 1x PBS for 5x5 minutes. ☐ ☐ ☐ ☐ ☐
2. Store the sections at 4°C in either 1x PBS (for short-term storage, <24 hrs) or 1x PBS Azide (for long-term storage, >24 hrs).

**DAB Staining**

**Chromogenic**

**Diaminobenzidine peroxidase reaction medium for BROWN reaction product**

* *30% Hydrogen peroxide (H2O2) 7.5 μl 15 μl*
* *DAB (Sigma) 12.5 mg 25 mg*
* *1x PBS 50 mL 100 mL*

**Diaminobenzidine peroxidase reaction medium for BLACK reaction product**

* *0.5% Cobalt(II) chloride (CoCl2) 1.5 mL 3 mL*
* *30% Hydrogen peroxide (H2O2) 7.5 μl 15 μl*
* *DAB (Sigma) 12.5 mg 25 mg*
* *1x PBS 48.5 mL 97 mL*

**Protocol**

1. Prepare a waterbath containing bleach to neutralize spills and to deactivate DAB after experiment is finished.
2. Prepare diaminobenzidine peroxidase reaction medium.
   1. BDA is detected with a black DAB reaction while CTB is detected with a brown DAB reaction.
3. Incubate the sections in the reaction medium until the reaction is considered complete – this can be from 10 seconds to 2 minutes.
4. Wash sections in 1x PBS for 3x10 minutes.