**Visualizing Biotinylated Dextran Amine (BDA) Protocol**

**Chromogenic or Fluorescent**

**Bleach Solution**

**(50% methanol & 1% hydrogen peroxide in PBS) 18 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*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Streptavidin type | Storage [ ] (%) | Dilution factor | Volume of reagent (μL) | Volume of 0.4% PBS-Tx (mL) |
| \_\_\_\_\_\_\_\_\_\_ | \_\_\_\_\_\_ | 1 : \_\_\_\_\_\_ | \_\_\_\_\_\_ | 20 |

e.g. in order to get a dilution of 1:250 for streptavidin with 50% concentration:

* Multiply the dilution factor (1/250) with the volume of PBS-Tx (20)
* Convert the resulting volume from mL to μL by multiplying it by 1000
* Correct for the storage concentration by multiplying the volume by 2 (because 100/50)

**Protocol**

*For each step: Make ~20 mL of solution per tray. Fill the wells up to around half-full so that the sections are fully submerged without being able to float out over the top. Make sure all sections are free-floating and not stuck to the sides. Put the tray on the rotator. Dry sections off between each wash by dabbing the tray on absorbent bench liner.*

1. Wash sections in 1x PBS for 5x5 minutes.
2. Bleach sections for 20 minutes.
3. Wash sections in 1x PBS for 5x5 minutes.
4. Incubate sections in streptavidin solution for 1 hour at room temp.
   1. For fluorescent injections: from this point on, cover sections with tinfoil to reduce light exposure to fluorescent-stained tissue.
5. Wash sections in 1x PBS for 5x5 minutes.
6. 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 Protocol**

**Chromogenic**

**Diaminobenzidine Peroxidase Reaction Medium**

**(for BROWN reaction product) 50 mL 100 mL**

* *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) 50 mL 100 mL**

* *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.