Visualizing Biotinylated Dextran Amine (BDA) Protocol
Chromogenic or Fluorescent

Bleach Solution
(50% methanol & 1% hydrogen peroxide in PBS) 18 mL

- *30% Hydrogen peroxide (H_2O_2)* *700 µL*
- *Methanol (CH_3OH)* *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

<u>For each step</u>: 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.
   a. 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 <u>BROWN</u> reaction product)	**50 mL**	**100 mL**
– *30% Hydrogen peroxide (H_2O_2)*	*7.5 µl*	*15 µl*
– *DAB (Sigma)*	*12.5 mg*	*25 mg*
– *1x PBS*	*50 mL*	*100 mL*

Diaminobenzidine peroxidase reaction medium

(for <u>BLACK</u> reaction product)	**50 mL**	**100 mL**
– *0.5% Cobalt(II) chloride ($CoCl_2$)*	*1.5 mL*	*3 mL*
– *30% Hydrogen peroxide (H_2O_2)*	*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.
   a. 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.