



# Neuroscience Molecular Detection and Stereology Core

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## Core B- Molecular Detection and Stereology Core

- Immunohistochemistry (IHC)
- in situ hybridization (ISH)
- Assistance in developing new protocols
- Training and production of high quality, reproducible molecular localization quantification in tissue and cell samples

## OBJECTIVE

The UAB Neuroscience Molecular Detection and Stereology Core (NMDSC) is designed to facilitate basic and clinical neuroscience research by NINDS-supported investigators.

The goal of the NMDSC is to assist in all aspects of immunohistochemistry (IHC) and in situ hybridization (ISH) from proper tissue collection and fixation methodology to tissue processing and paraffin embedding. From this point, the NMDSC is available to assist with antibody specific protocol development and training of researchers, students, and staff in the varied detection systems available at the NMDSC.

Following staining optimization the core has two imaging systems available for quantification and analysis. These include StereoInvestigator software for unbiased stereology and NeuroLucida software for neuronal analysis. The core provides training and experimental planning for these systems.

For additional resources and information check out our website or like us on Facebook:

[www.neuroscience.uab.edu/coreb.htm](http://www.neuroscience.uab.edu/coreb.htm)

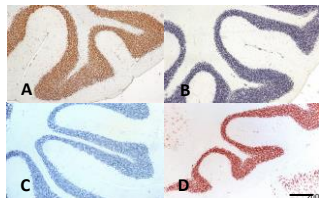
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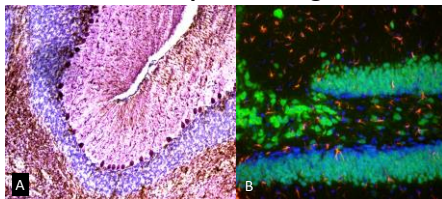
Phone 996-6556

## Chromogenic Detection



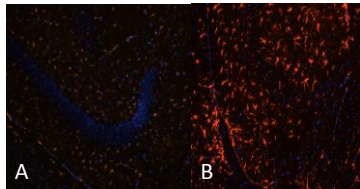
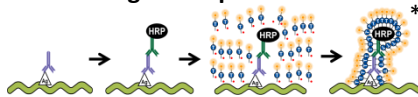
IHC detection of neurons with mouse anti-NeuN of the cerebellum in formalin fixed, paraffin embedded mouse brain utilizing HRP-dependent chromogens. (A) DAB, (B) DAB-Nickle, (C) tetramethylbenzide, and (D) 3-amino-9-ethylcarbazole.

## Multiple Labeling



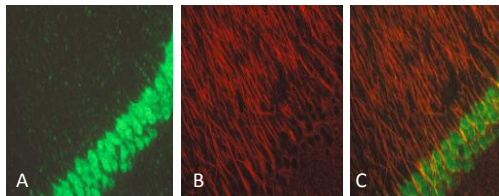
Various detection methods for multiple labeling. A) Chromogenic IHC detection of Rabbit anti-GFAP (brown), rabbit anti-Calbindin on (purple), and mouse anti-NeuN (blue). B) Mouse hippocampus double stained with ms alpha-NeuN (neurons), Rbt alpha-GFAP (astrocytes) and Bisbenzimidazole (nuclei).

## Signal Amplification



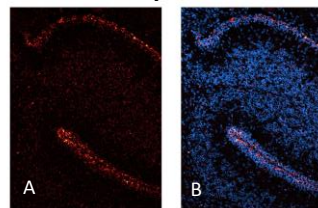
Utilizing Tyramide signal amplification (TSA) methods increases sensitivity, reduces background, and lowers cost by reducing amount of antibody required. A) Rabbit anti-GFAP 1:1000 detected with Cy3 conjugated secondary. B) Rabbit anti-GFAP 1:10,000 detected with TSA-Cy3.

## Dilutional Neglect



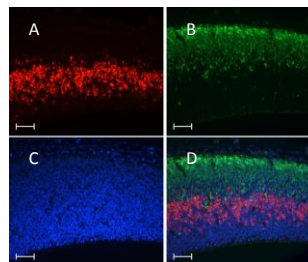
Detection of multiple antibodies raised in the same species with conventional and TSA detection in mouse hippocampus. A) Ms alpha-NeuN diluted to undetectable level by conventional methods. TSA increases sensitivity and allows detection. B) Ms alpha-MAP2 by conventional methods. C) Ms alpha-NeuN and Ms alpha-MAP2 merged.

## In Situ Hybridization



Mouse cerebellum hybridized with a histone (H4) dCTP digoxigenin-labeled antisense probe. A) Anti-digoxigenin antibody was used to detect the hybridized probe and signal was amplified using TSA-Plus (red). B) Nuclei were counterstained with DAPI (blue).

## ISH/IHC Multi-Labeling



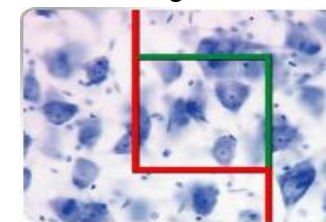
A) *In Situ* hybridization for histone H4 followed by B) immunohistochemical detection of MAP2. C) Hoechst dye. D) merged

## Stereologic/Morphometric Analysis



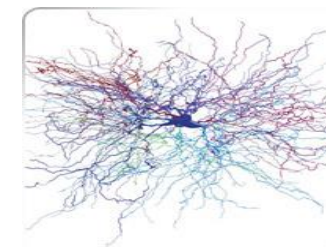
Two BX51 Olympus microscopes with Microfire optical cameras for bright field and fluorescent imaging.

## StereInvestigator Software



Each system has StereoInvestigator software installed for unbiased cell counting and quantification of IHC staining. In addition the software is capable of calculating Cavalieri volumes and cell areas.

## NeuroLucida Software



The NeuroLucida software provides the ability to do neuron tracing, anatomical mapping, and neuronal tree analyses

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\* Figure courtesy of Perkin Elmer