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Glutamic Acid Decarboxylase-67, IHC-Paraffin Staining Report

Target Antigen:	**GAD67**
Catalog or ID #:	MAB5406
Host and Isotype:	Mouse IgG1 and 2_a
Purity and Method:	Purified immunoglobulin
Concentration and Quantity:	1 mg/mL
Storage Conditions:	2-4.0^{o}C refrigerated
Species Reactivity:	Human, rat and mouse. Reactivity with other species has not been determined
IHC-Paraffin Fixation Tested:	10% neutral Buffered Formalin
IHC-Paraffin Titer:	1:20 to 1:50 for 1 hour incubation or 1:50 for 1:100 over night incubation at 4.0^{o}C
IHC-Paraffin Pretreatment(s):	Recommend no Heat Induced Epitope Retrieval (HIER).
IHC-Paraffin Protocol:	**IHC-Select, HRP-DAB** (Brief Outline): Blocking Reagent for 10 min. Primary Antibody (or Negative Control Reagent) for Over Night incubation at 4.0^{o}C. Biotinylated Secondary Antibody for 10 min. Streptavidin-HRP for 10 min. DAB for 10 min. Hematoxylin for 1 min.
Positive Tissue(s):	GABAergic neurons in the higher regions of the mouse, rat and human brain.
Subcellular Location(s):	Cytoplasmic staining
Negative Tissue(s):	Many different neural tissues.
Comments and References:	Staining is as expected since immunoreactivity was seeing as cytoplasmic staining in the following higher function brain origins: Most dramatic staining was seeing in the reticular thalmic nucleus (RTN) and the lateral septal nucleus as well as lateral cerebellar nucleus (Lat) all the way to lateral vestibular nucleus (Lve) . Scatter staining from area A1 (noradrenergic cell field) all the way to the extreme capsule, hippocampus (CA1 to CA3). Other regions include medial preoptic nucleus, median eminence, ventromedial nucleus, suprachiasmatic nucleus, medial septal nucleus and cingulated cortex.

IHC – Paraffin Staining Examples:

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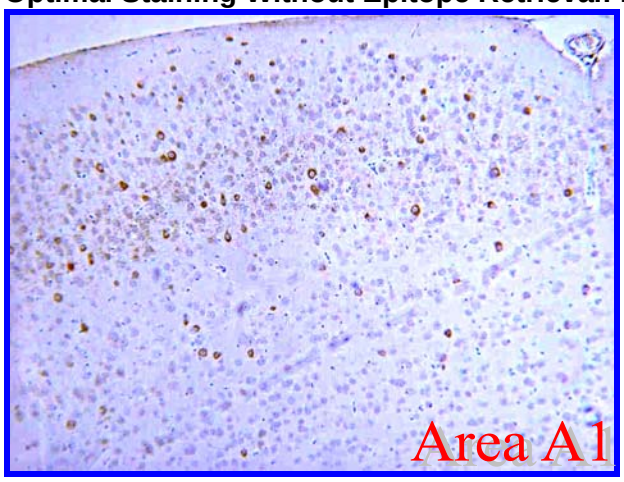
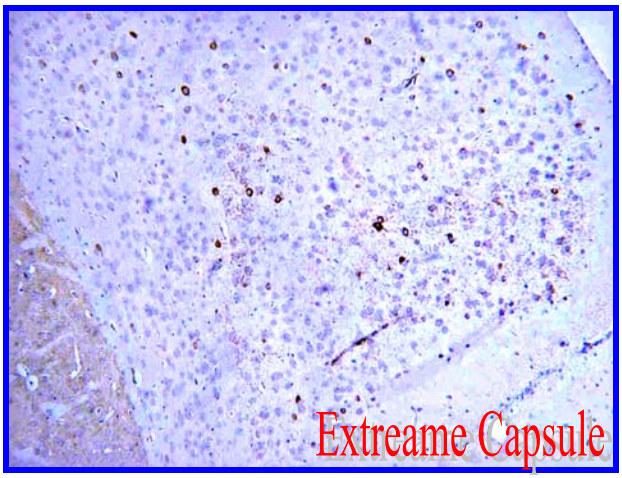
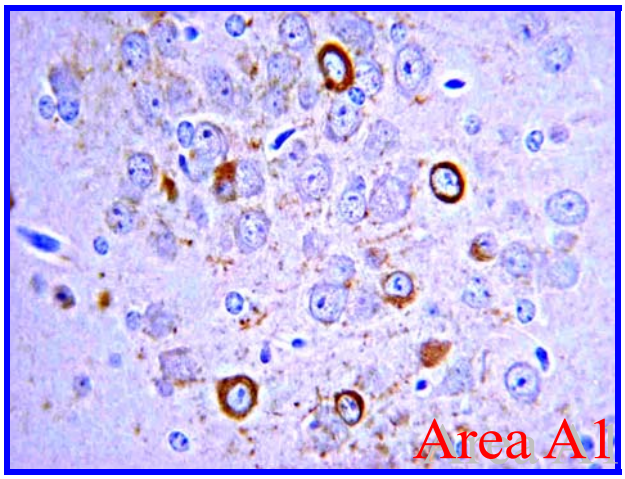
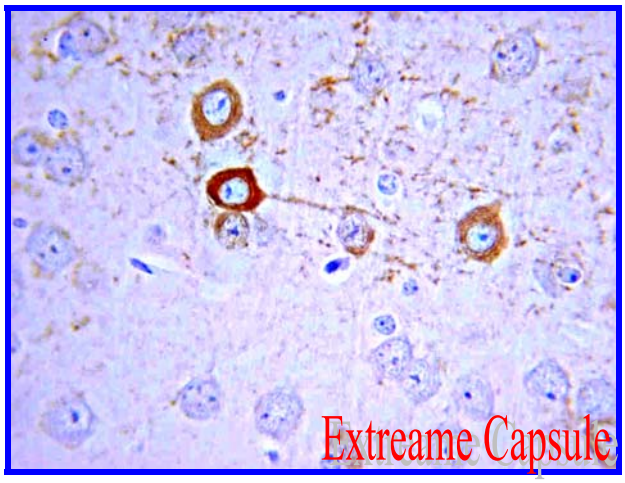
Optimal Staining Without Epitope Retrieval: Mouse Brain





Representative staining pattern and morphology of GAD67 (MAB5406). Scatter staining is seeing from area A1 (noradrenergic cell field) all the way to the extreme capsule. No Epitope retrieval was necessary. Monoclonal Ab. diluted to 1:50 (O/N at 4.0^0 C), IHC-Select Detection with HRP-DAB. Immunoreactivity is seeing as cytoplasmic staining (brown). Low Mag.





Representative staining pattern and morphology of GAD67 (MAB5406). Scatter staining is seeing from area A1 (noradrenergic cell field) all the way to the extreme capsule. No Epitope retrieval was necessary. Monoclonal Ab. diluted to 1:50 (O/N at 4.0^0 C), IHC-Select Detection with HRP-DAB. Immunoreactivity is seeing as cytoplasmic staining (brown). High Mag.

IHC – Paraffin Staining Examples:

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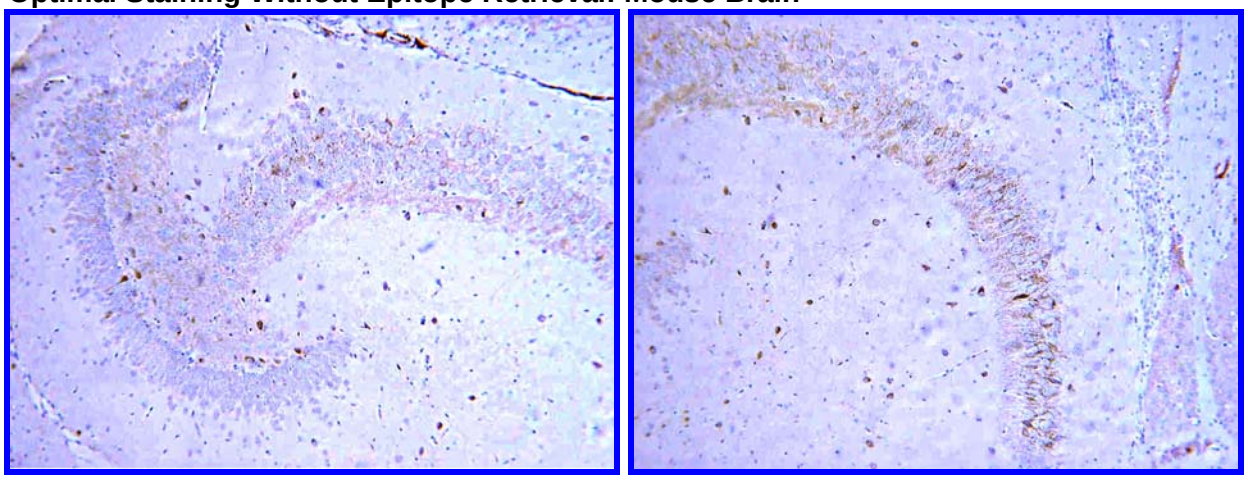
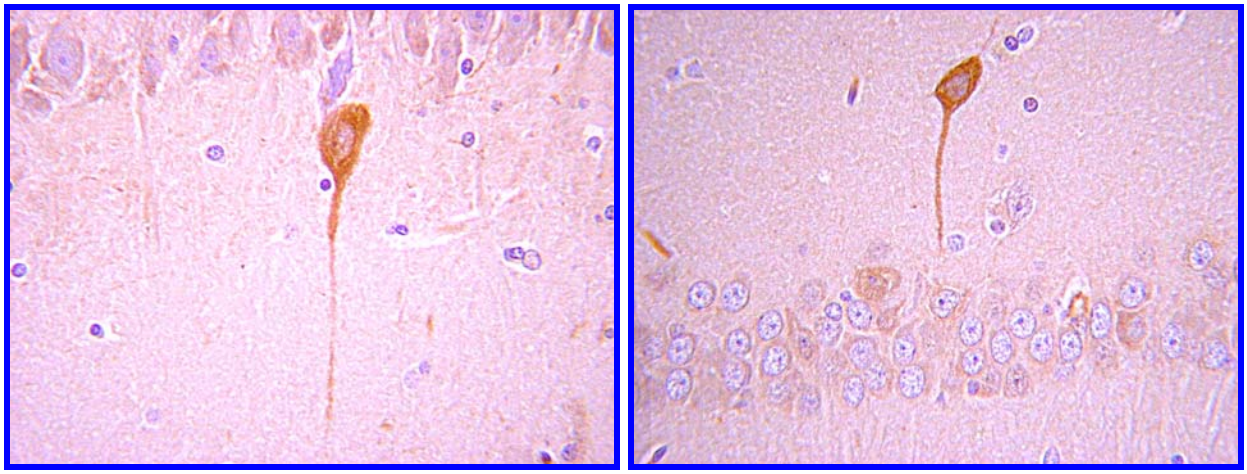
Optimal Staining Without Epitope Retrieval: Mouse Brain



Representative staining pattern and morphology of GAD67 (MAB5406) in the CA1, CA2, CA3 field of the hippocampus and polymorph layer. In the lower magnification image all brown spots are positively staining cells. No Epitope retrieval was necessary. Monoclonal Ab. diluted to 1:50 (O/N at 4.0^0 C), IHC-Select Detection with HRP-DAB. Immunoreactivity is seeing as cytoplasmic staining (brown). Low Mag.



Representative staining pattern and morphology of GAD67 (MAB5406) in the CA1, CA2, CA3 field of the hippocampus and polymorph layer. In the lower magnification image all brown spots are positively staining cells. No Epitope retrieval was necessary. Monoclonal Ab. diluted to 1:50 (O/N at 4.0^0 C), IHC-Select Detection with HRP-DAB. Immunoreactivity is seeing as cytoplasmic staining (brown). High magnification of image above.

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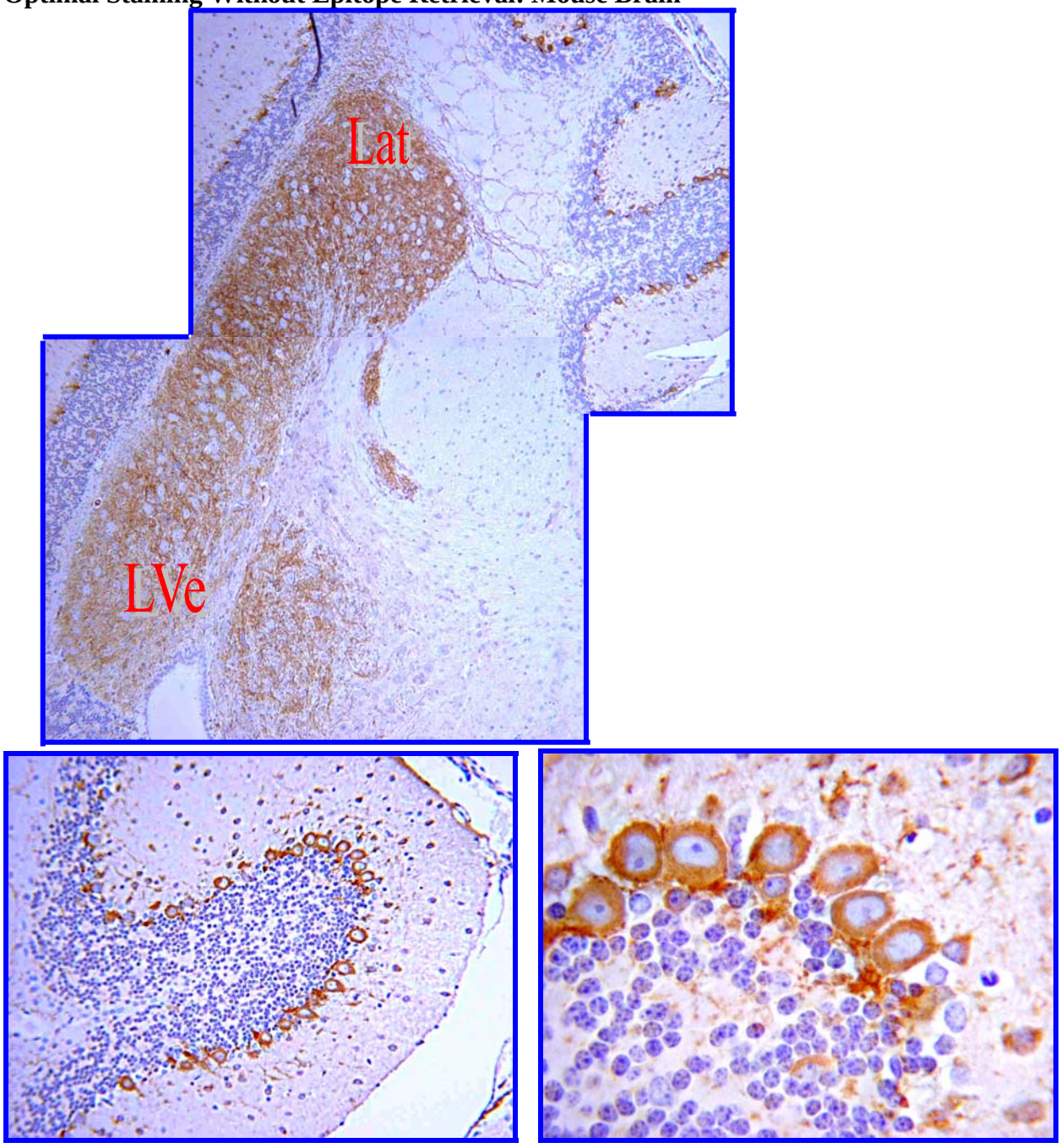
IHC – Paraffin Staining Examples:
Optimal Staining Without Epitope Retrieval: Mouse Brain



Representative staining pattern and morphology of GAD67 (MAB5406) in the lateral cerebellar nucleus (Lat) all the way to lateral vesticualr nucleus (Lve). In the lower magnification image all brown color/spots are positively staining cells. In the higher magnification Purkinje cell involving a postsynaptic staining pattern as well as an axon-staining pattern is visible. No Epitope retrieval was necessary. Monoclonal Ab. diluted to 1:50 (O/N at 4.0^0 C), IHC-Select Detection with HRP-DAB. Immunoreactivity is seeing as cytoplasmic staining (brown).

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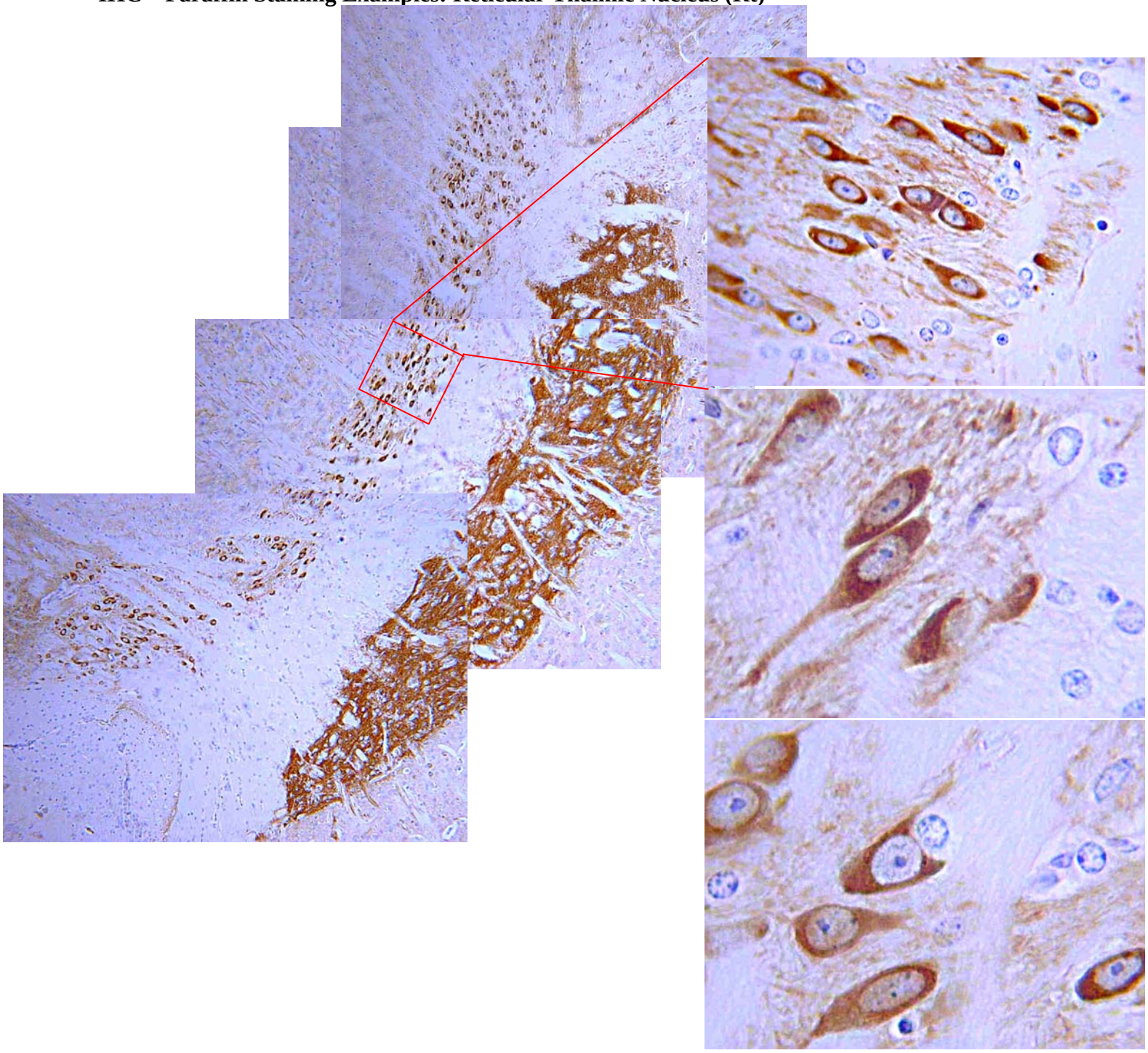
IHC – Paraffin Staining Examples: Reticular Thalmic Nucleus (Rt)



Representative staining pattern and morphology of GAD67 (MAB5406) in the reticular thalamic nucleus (Rt) In the lower image composite (showing a large region) all brown color/spots are positively staining cells. For staining clarity, higher magnification images are showed on the right.