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Rat HSD3B1+ HSD3B2 Ready-To-Use IHC Kit

Cat.No: IHC0187R
Applications: **IHC-P**
Reactivity: Rat
Size: 50T
Assay type: Immunohistochemistry
Sample type: FFPE tissue
General Information:

Number	Component	Size	Concentration	Storage
1	PBS Buffer (powder)	2 L×2	20x	RT
2	Antigen Retrieval Buffer	20 ml	100x	2-8°C
3	Endogenous Peroxidase Blocking Buffer	3 ml	RTU	2-8°C, protect from light
4	Blocking Buffer	3 ml	RTU	2-8°C
5	Primary Antibody (Rat HSD3B1+ HSD3B2 Rabbit pAb)	6 ml	RTU	2-8°C
6	Secondary Antibody (HRP-Goat anti-Rabbit IgG pAb)	6 ml	RTU	2-8°C
7	Chromogen Component A	0.3 ml	RTU	-20°C,protect from light
8	Chromogen Component B	0.3 ml	RTU	-20°C
9	Counter Staining Reagent	5 ml	RTU	RT
10	Mounting Media	5 ml	RTU	RT
11	Control slide (Rat adrenal gland)	1 slide	RTU	RT
12	Datasheet	1 copy		

Storage and Stability: Please store components at the temperatures indicated on the individual tube labels. The kit is stable for 6 months from the date of receipt.

Immunohistochemistry Protocol:

1. **Deparaffinization And Rehydration**

Immerse slides in fresh xylene for 15 minutes and then repeat two more times using separate containers. Immerse slides sequentially in 100%, 95%, 90%, 80%, and 70% ethanol solutions for 5 minutes each. Rinse slides 3 times with distilled water for 5 minutes each.

2. **Antigen Retrieval**

Add 100× **Antigen Retrieval Buffer** into distilled water to prepare a 1×solution. Boil slides in 1×solution at 95°C-100°C for 15 minutes. Move the slides to 1×solution at room temperature (RT) and allow them to stand for 20 minutes. Rinse 3 times with **PBS Buffer** (dissolve the powder in 2L distilled water) for 5 minutes each.

3. **Block Endogenous Peroxidase**

Drain the liquid off the slides and then use a hydrophobic IHC pen to draw circles on the slides around tissue sections. Add 2-4 drops of **Endogenous Peroxidase Blocking Buffer** directly on slides, covering the whole tissue and block slides for 15 minutes at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

4. **Serum Blocking**

Block with 2-4 drops of **Blocking Buffer** for 20 minutes at RT.

5. **Primary Antibody Incubation**

Drain blocking buffer from slides. Incubate slides with 2-4 drops of **Rat HSD3B1+ HSD3B2 Rabbit pAb** overnight at 4°C or 1-2 hours at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

6. **Secondary Antibody Incubation**

Incubate slides with 2-4 drops of **HRP-Goat anti-Rabbit IgG pAb** for 1-2 hours at RT. Rinse slides 3 times with **PBS Buffer** for 5 minutes each.

7. **Signal Development**

Remove residual liquid around the tissue section. Add 50ul fresh **DAB Buffer** (**Chromogen Component A : Chromogen Component B : PBS Buffer=1:1:18**) to cover the tissue. Monitor the reaction under the microscope until a brown color is visible (approximate 3-5 minutes at RT). Stop reaction immediately by rinsing with distilled water. Rinse slides 3 times with distilled water for 5 minutes each.

8. **Counterstain**

Counterstain with an appropriate amount of **Counter Staining Reagent** for 3-5 minutes at RT. Rinse slides with distilled water for 5 minutes. Use 2-4 drops of **Differentiation reagent** to cover the tissue for 30 seconds. Rinse slides twice with distilled water for 5 minutes each.

9. **Dehydration Sheet**

Immerse slides sequentially in 70%, 80%, 90%, 95%, and 100% ethanol for 5 minutes each at RT. Immerse slides in 2 changes of fresh xylene, 15 minutes each. Drop some **Mounting Media** on the tissue. Mount coverslips.

Notes:

1. The positive control slide provided in the kit allows you to be sure that the experimental set-up is working properly.
2. Do not allow slides to dry at any time during this procedure.

3. Please don't replace the matching reagents in this product with other manufacturers' products.

4. As DAB is a carcinogen, please take necessary precautions.

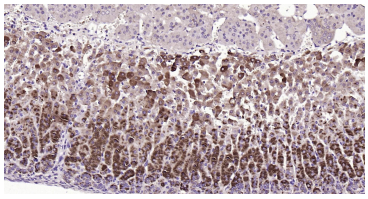
5. PBS (reagent 1) can be stored for one week at 4°C after preparation; The antigen retrieval buffer (1×reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

Please cite this product as " IHC0187R, Bioss Antibodies". Citation example: " Rat Tissue sections using HSD3B1 IHC Kit (IHC0187R, Bioss Antibodies) were stained for HSD3B1 according to the manufacturer's instructions."

Introduction:

Amyloid beta peptide (Abeta/Beta-amyloid) is the major constituent of amyloid plaques in the brains of individuals afflicted with Alzheimer's disease. Abeta peptide is 40-43 amino acids long and generated from the beta-amyloid precursor protein (beta APP) in a two-step process. The first step involves cleavage of the extracellular, amino-terminal domain of beta APP. Protein cleavage is performed by an aspartyl protease, beta-secretase (BACE) which is synthesized as a propeptide and must be modified to the mature and active form by the prohormone convertase, furin. Beta APP cleavage by the mature form of BACE results in the cellular secretion of a segment of beta APP, and a membrane-bound remnant. The remnant protein is processed by another protease, gamma-secretase. Gamma-secretase cleaves an intra-membrane site in the carboxyl-terminal domain of beta APP, thus generating the amyloid beta peptide. Gamma-secretase is believed to be a multi-subunit complex containing presenilin-1 and 2 as central components. The transmembrane glycoprotein, nicastrin, is associated with presinilins and has been found to bind to the carboxyl-terminus of beta APP and helps to modulate the production of the amyloid beta peptide. Abeta is an extracellular filamentous protein component of amyloid cores, neuritic plaques and is also found as a deposit in neurofibrillary tangles. Alzheimer's disease, the most common cause of senile dementia, is characterized by abnormal filamentous protein deposits in the brain. Beta amyloid deposits are also detected in Lewy body dementia, Down's syndrome, amyloidosis (Dutch type), cerebroarterial amyloidosis (cerebral amyloid angiopathy) and in the Guam Parkinson-Dementia complex.

Validation Data



Immunohistochemical analysis of paraffin embedded rat adrenal glands tissue slide using IHC0187R (Rat HSD3B1+ HSD3B2 IHC Kit).