NEUROMICS

NeuroD1 Data Sheet

Catalog Number:	GT15231	**Host:**	Goat
Product Type:	Affinity purified	**Species Reactivity:**	Human
Immunogen Sequence:	Purified, NS0-derived, recombinant human CUB Domain Containing Protein 1 isoform 1 (rhCDCP1) extracellular domain	**Format:**	Liquid 1mg/ml Phosphate-buffered saline (PBS) with 5% trehalose.
Applications:	Immunocytochemistry : 5-15 µg/mL (cultured cells) Western Blot: 1.0 µg/mL		
	Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.		
Storage:	Antibody can be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. The antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. *Avoid repeated freeze-thaw cycles.*		

Application Notes

Specificity
Useful for immunocytochemistry and western blots. Approximately 5% cross reactivity with recombinant human NeuroD2 is observed.

Description/Data:
NeuroD1 (Neurogenic Differentiation factor 1, also known as BETA2 (beta-cell E-box transactivator 2) is a 40kDa protein, a member of the bHLH family. NeuroD1 is a homolog of the Drosophila atonal gene. NeuroD1 is widely expressed during development in the mammalian brain and pancreas where it acts as a transcriptional activator, and binds to insulin gene E-box. NeuroD1 has also been reported to be a differentiation factor in neurogenesis. Changes in NeuroD1 gene expression have been linked to diabetes mellitus type II and maturity-onset diabetes of the young type VI. NeuroD1 expression can be induced by glucose in beta cells.

Immunocytochemistry
Reagents Required
- Primary Antibodies
- Blocking buffer: 10% normal donkey serum, 0.3% Triton™ X-100
- DAPI (4',6-diamidino-2-phenylindole) solution: add 1 µL of 14.3 mM stock for every 5 mL of PBS. Store any unused DAPI at 2-8 °C, wrapped in aluminum foil
- Deionized H2O
- Dilution buffer: PBS (1x), 1% bovine serum albumin (BSA), 1% normal donkey serum, 0.3% Triton X-100, and 0.01% sodium azide
- Anti-fade mounting medium (i-BRITE Plus)
- Fluoro-conjugated secondary antibody
- PBS (1x): 0.137 M NaCl, 0.05 M NaH2PO4,
- pH 7.4
- Wash and antibody dilution buffer: 0.1% BSA in PBS (1x)

Materials
- Cell-covered coverslips in a 6- or 24-well plate
- Fine tweezers

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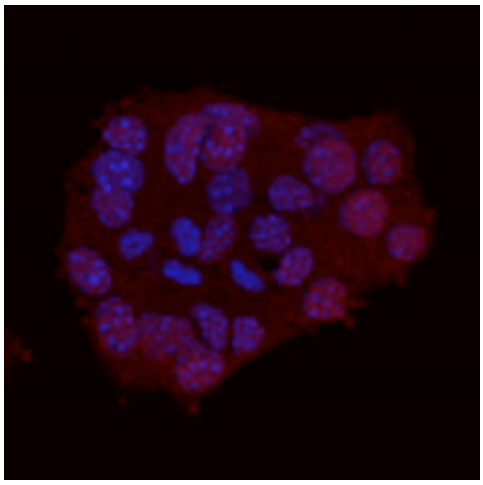
Procedure
Note: This protocol is optimized for cells grown on coverslips in a 6- or 24-well plate but can be adapted accordingly.

1. Wash the coverslips containing the fixed cells two times in 400 µL of wash buffer
2. Block non-specific staining by adding 400 µL of blocking buffer and incubate for 45 minutes at room temperature.
3. Remove blocking buffer. No rinsing is necessary.
4. Dilute the NeuroD1 antibody as recommended. Incubate at room temperature for 1 hour. Alternatively, incubate overnight at 2-8 °C.
5. Wash two times in 400 µL of wash buffer.
6. Dilute the secondary antibody in dilution buffer according to the manufacturer's instructions. Add 400 µL to the wells and incubate at room temperature for 1 hour in the dark. From this step forward samples should be protected from light.
7. Rinse two times in 400 µL of wash buffer.
8. Add 300 µL of the diluted DAPI solution to each well and incubate 2-5 minutes at room temperature. DAPI binds to DNA and is a convenient nuclear counterstain. It has an absorption maximum at 358 nm and fluoresces blue at an emission maximum of 461 nm.
   Note: DAPI counterstain can obscure visualization of targets localized in cell nuclei.
9. Rinse once with PBS and once with water.
10. Carefully remove the coverslips from the wells and blot to remove any excess water. Dispense 1 drop of anti-fade mounting medium onto the microscope slide per coverslip. Mount the coverslip with the cells facing towards the microscope slide.
11. Visualize using a fluorescence microscope and filter sets appropriate for the label used. Slides can also be stored in a slide box at < -20 °C for later examination.

Image: NeuroD1 staining of immersion fixed beta TC-6 mouse beta cell insulinoma cell line at 10 µg/mL for 3 hours at room temperature. Cells were stained using the Fluoro-labeled ™ 557-conjugated Anti-Goat IgG Secondary Antibody (red) and counterstained with DAPI (blue)



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