Revised: 23–April–2001

Anti-Human Neuronal Protein HuC/HuD (anti-HuC/D)

Quick Facts

Storage upon receipt:

- –20°C
- Desiccate
- Protect from light (A-21275, A-21276)

Introduction

Anti-Hu antibodies were originally isolated from a patient with paraneoplastic encephalomyelitis, a rare condition of selective neural tissue injury associated with small cell lung carcinoma.[1] The antibodies bind specifically to antigens present exclusively in neuronal cells and are thus useful as markers of neuronal cells in tissue. The Hu antigen is an RNA-binding protein of the embryonic lethal abnormal visual (Elav) family. Molecular Probes' anti-human neuronal protein HuC/HuD, mouse monoclonal antibody 16A11 (isotype, $IgG_{2b,\kappa}$), recognizes the Elav family members HuC,[2] HuD [1] and Hel-N1,[3] which are all neuronal proteins. Anti-HuC/D monoclonal 16A11 does not recognize HuR, another Elav family member that is present in all proliferating cells.[4] The antibody has been shown to specifically label neuronal cells in zebrafish,[5] chick,[6] canaries [7] and humans [8] and is likely to label neuronal cells in most vertebrate species. Labeling is visible early in development, at about the time that the neurons leave the mitotic cycle.[8]

The unlabeled anti-HuC/D mouse monoclonal antibody 16A11 is generally used in conjuction with a secondary antibody, such as labeled goat anti-mouse IgG. Molecular Probes also provides biotin-XX, Alexa Fluor® 488 and Alexa Fluor 594 conjugates of anti-HuC/D monoclonal 16A11. The biotin-XX labeled antibody can be detected by using fluorescently labeled avidin, streptavidin or NeutrAvidin™ conjugates. The biotin-XX– and the Alexa Fluor–labeled conjugates of anti-HuC/D are preferred for staining mouse tissue, to avoid the possible background staining from the use of a secondary anti-mouse IgG antibody.

***Table 1**. Molecular Probes' mouse anti-human neuronal protein HuC/HuD antibodies.**

Catalog #	Label	Abs †	Em †
A-21271	unlabeled	NA	NA
A-21272	biotin-XX	NA	NA
A-21275	Alexa Fluor 488	495	519
A-21276	Alexa Fluor 594	590	617

***** Clone 16A11. † Approximate absorption (Abs) and fluorescence emission (Em) maxima in nm.

Materials

The unlabeled anti-human neuronal protein HuC/HuD, mouse monoclonal 16A11, and the biotin-XX and Alexa Fluor conjugates are all provided in a 100 µg unit size. Upon receipt the lyophilized antibodies should be stored desiccated at -20°C or lower. When properly stored, these products are stable for at least one year.

To prepare stock solutions, reconstitute the antibodies in 0.5–1 mL of phosphate-buffered saline, pH 7.4, containing 1% bovine serum albumin (BSA). Store the solutions for up to two weeks at 4°C with the addition of 2 mM sodium azide. For longer storage, divide solutions into single-use aliquots and freeze at 20°C. AVOID REPEATED FREEZING AND THAWING.

Applications

Because protocols vary with application, the appropriate dilution of the anti-HuC/D antibodies should be determined empirically. We recommend trying an initial concentration of 5–20 µg/mL for immunohistochemistry, 1–5 µg/mL for Western blotting and 1–10 µg/mL for solid-phase immunoassay. For best results in immunohistochemistry, we have found that an "antigen-retrieval" procedure is required. Below, as an example, we outline our procedure for staining 16 µm cryosections of mouse embryos with the biotin-XX conjugate of anti-HuC/D mouse monoclonal antibody.

1. Rehydrate the sectioned material in phosphate-buffered saline (PBS) for 40 minutes.

2. Perform antigen retrieval by submersing the slide in 50 mM Tris, pH 8.0, in a staining jar and then placing this jar in a boiling-water bath for 30 minutes. Add boiling chips to the boiling-water bath to prevent splattering.

3. Cool the slide to room temperature.

4. Wash the specimen for 10 minutes in PBS plus 0.1% Triton® X-100.

5. Wash the specimen twice for 5 minutes each in PBS.

6. Block the specimen for 30 minutes in BlockAid blocking solution (B-10710) or in another suitable blocking formulation.

7. Incubate the specimen overnight at 4°C in 10 µg/mL of the biotin-XX conjugate of anti-HuC/D in PBS plus 1% BSA. We recommend using an immunohistochemical grade of BSA.

8. Wash the specimen three times for 10 minutes each in PBS plus 1% BSA.

9. Incubate the specimen 20 minutes at room temperature in 5 µg/mL of an avidin labeled with the dye, hapten or enzyme of your choice in PBS plus 1% BSA. ***Note*:** If using the unlabeled anti-HuC/D mouse monoclonal antibody, then a labeled anti-mouse IgG, rather than labeled avidin, should be used for secondary detection.

10. Wash the specimen twice for 10 minutes each in PBS.

11. Counterstain as desired.

12. Apply a suitable mounting medium and cover the specimen with a glass coverslip. We recommend using the ProLong® Antifade Kit (P-7481) for mounting.

References

1. Cell 67, 325 (1991); **2.** Neurology 45, 544 (1995); **3.** Mol Cell Biol 13, 3494 (1993); **4.** J Neuroimmunol 92, 152 (1994); **5.** Dev Genet 18, 11 (1996); **6.** Development 124, 3449 (1997); **7.** J Neurobiol 28, 82 (1995); **8.** J Neurobiol 25, 143 (1994).

Product List

Current prices may be obtained from our Web site or from our Customer Service Department.

Cat #	Product Name	Unit Size
A-21271	anti-human neuronal protein HuC/HuD, mouse monoclonal 16A11 (anti-HuC/D)	100 µg
A-21272	anti-human neuronal protein HuC/HuD, mouse monoclonal 16A11, biotin-XX conjugate (anti-HuC/D, biotin conjugate)	100 µg
A-21275	anti-human neuronal protein HuC/HuD, mouse monoclonal 16A11, Alexa Fluor® 488 conjugate (Alexa Fluor® 488 anti-HuC/D)	100 µg
A-21276	anti-human neuronal protein HuC/HuD, mouse monoclonal 16A11, Alexa Fluor® 594 conjugate (Alexa Fluor® 594 anti-HuC/D)	100 µg

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — **www.probes.com** — for the most up-to-date information

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