

Ordering Information

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HGNC name: FBL

RRID: Pending

Immunogen: Recombinant full length human sequence expressed in and purified from *E. coli*.

Format: Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM Na₂S₂O₃

Storage: Shipped on ice. Stable at 4°C for one year, for longer term store at -20°C

Recommended dilutions:

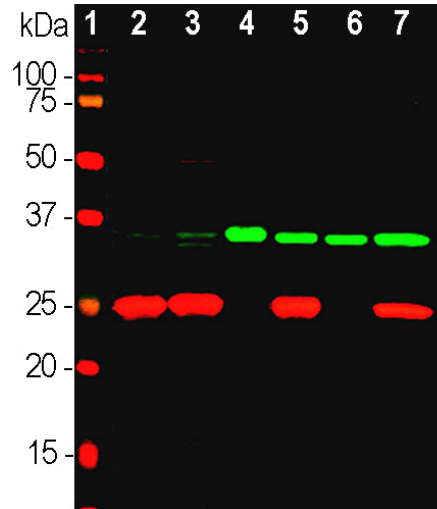
Western blot: 1:500-1:1,000.

ICC/IF and IHC: 1:1,000-1:5,000.

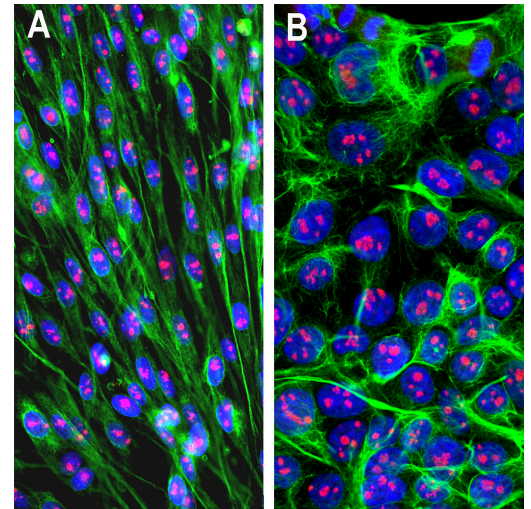
References:

1. Aris JP and Blobel G. cDNA cloning and sequencing of human fibrillarin, a conserved nucleolar protein recognized by autoimmune antisera. *Proc. Natl. Acad. Sci.* 88:931-935 (1991).
2. Ochs RL, Lischwe MA, Spohn WH, Busch H. Fibrillarin: a new protein of the nucleolus identified by autoimmune sera. *Biol Cell* 54:123-133 (1985).
3. Aris JP and Blobel G. Identification and characterization of a yeast nucleolar protein that is similar to a rat liver nucleolar protein. *J. Cell Biol.* 107:17-31 (1988).
4. Newton K, Petfalski E, Tollervey D, Caceres JF. Fibrillarin is essential for early development and required for accumulation of an intron-encoded small nucleolar RNA in the mouse. *Mol Cell Biol.* 23:8519-8527 (2003).

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
Western blot, ICC/IF, IHC	Mouse	IgG1	34.5kDa	Hu, Rt, Ms



Western blot analysis of different tissue and cell line lysates using mouse mAb to fibrillarin MCA-4A4, dilution 1:2,000, in green: [1] protein standard (red), [2] rat whole brain, [3] mouse whole brain, [4] NIH-3T3 cells, [5] HEK293, [6] HeLa, and [7] SH-SY5Y cells. Strong band at ~35kDa corresponds to the fibrillarin protein seen in all cell line lysates. A much weaker band is seen in tissue lysates since fibrillarin is more heavily expressed in rapidly dividing cells. The blot simultaneously was probed with rabbit pAb to UCHL1, *RPCA-UCHL1*, dilution 1:3,000, in red, revealing the ~25kDa UCHL1 protein in lysates of tissues containing neurons or cells with neuronal properties.



Immunofluorescent analysis of (A) C6 rat glioma cells and (B) HEK293 human embryonic kidney cells stained with mouse mAb to fibrillarin, MCA-4A4, dilution 1:1,000 in red, in both cases costained with chicken pAb to vimentin, *CPCA-Vim*, dilution 1:10,000 in green. The blue is DAPI staining of nuclear DNA. The MCA-4A4 antibody detects fibrillarin protein localized in nucleoli while the *CPCA-Vim* antibody produces strong staining of cytoplasmic intermediate filaments.

Background: Nop1p was originally identified as a nucleolar protein of bakers yeast, *Saccharomyces cerevisiae* (accession P15646). The Nop1p protein is 327 amino acids in size (34.5kDa), is essential for yeast viability, and is localized in the nucleoli (1). The systematic name for *S. cerevisiae* Nop1 is YDL014W, and it is now known to be part of the small subunit processome complex, involved in the processing of pre-18S ribosomal RNA. Nop1p is the yeast homologue of a protein apparently found in all eukaryotes and archae generally called fibrillarin. Fibrillarin/Nop1p is extraordinarily conserved, so that the yeast and human proteins are 67% identical, and the human protein can functionally replace the yeast protein. This means that suitably cross-reactive antibodies to Nop1p/fibrillarin, like MCA-4A4, can be used to reveal nucleoli and study fibrillarin/Nop1p in all eukaryotes and archae tested to date.

Human fibrillarin has been characterized (accession P22087) and the human fibrillarin gene is located on chromosome 19 (19q13.1). Fibrillarin/Nop1p proteins have been cloned and sequenced from several other species (e.g. Mouse, accession P35550, *Xenopus* accession P22232, *C. elegans* accession Q22053, and *S. pombe* accession P35551). The N terminal ~80 amino acids contain multiple copies based on the peptide RGG, or arginine-glycine-glycine, sometimes referred to as GAR repeats, characteristic of the GAR family of molecules. The remaining ~240 amino acids consist of the so called fibrillarin domain. A fibrillarin homologue has also been identified in the genome of the archaean *Methanococcus* (accession NC_000909). This protein lacks the RGG rich N-terminal extension but is clearly homologous to the other

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Abbreviation Key:

mAb—Monoclonal Antibody pAb—Polyclonal Antibody WB—Western Blot IF—Immunofluorescence ICC—Immunocytochemistry IHC—Immunohistochemistry E—ELISA Hu—Human Mo—Monkey Do—Dog Rt—Rat Ms—Mouse Bo—Cow Po—Pig Ho—Horse Ch—Chicken Dr—*D. rerio* Dm—*D. melanogaster* Ce—*C. elegans* Sc—*S. cerevisiae* Sa—*S. aureus* Ec—*E. coli*.

sequences throughout all of the fibrillarin domain. The 3D structure of this molecule has been determined and shown to consist of 2 extended β -sheets flanked by α -helices (Medline link). Patients with the autoimmune disease scleroderma often have strong circulating autoantibodies to a ~34kDa protein which was subsequently found to be fibrillarin. Recent studies show that knock out of the fibrillarin gene in mice results in embryonic lethality, although mice with only one functional fibrillarin/Nop1p gene were viable (3).

This antibody is becoming widely used as a convenient marker for nucleoli in a wide variety of species (e.g. 4-6). The HGNC name for this protein is FBL. To raise the MCA-4H4 antibody, mice were injected with full length recombinant human fibrillarin. Reference 2 below describes the characterization of D77, an antibody very similar but not identical to MCA-4A4.