

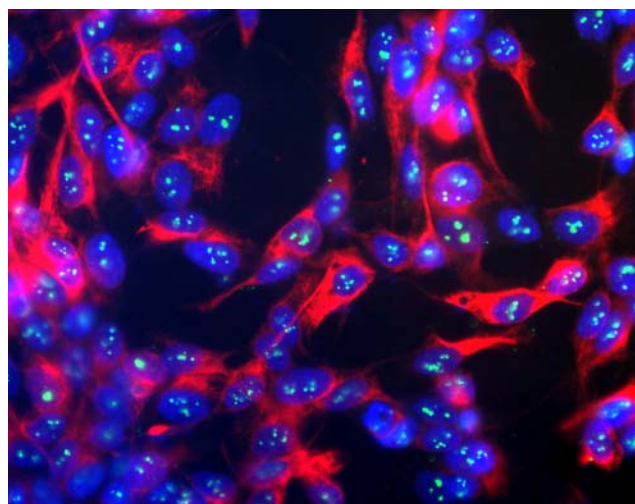
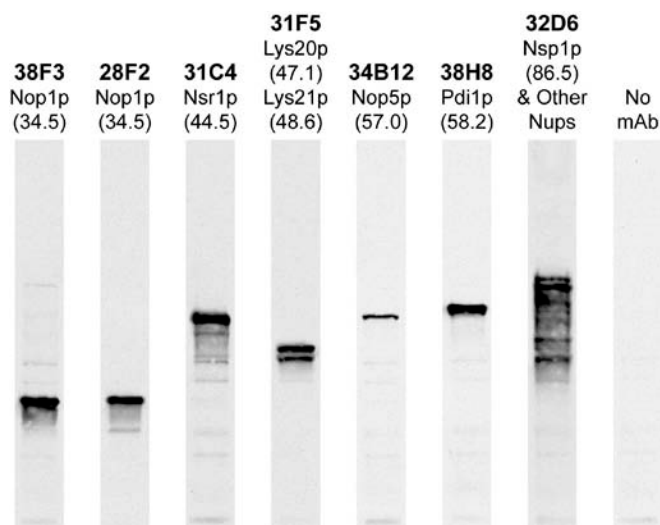
Catalogue# MCA-38F3: Monoclonal Antibody 38F3 to Fibrillarin/Nop1p

The Immunogen: 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 archea 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-38F3, can be used to reveal nucleoli and study fibrillarin/Nop1p in all eukaryotes and archea 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.

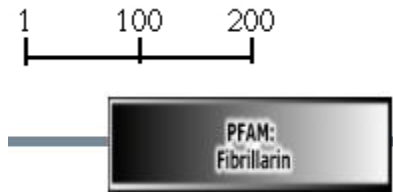
A fibrillarin homologue has also been identified in the genome of the archaean *Methanococcus* ([accession NC_000909](#)). This protein lacks the RGG rich N-terminal extension but is clearly homologous to the other sequences throughout all of the fibrillarin domain. The 3D structure of this molecule has been determined and shown to consist of 2 extended β -sheets flanked by α -helixes ([Medline link](#)). Patients with the autoimmune disease scleroderma often have strong circulating autoantibodies to a ~34 kDa 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](#).



Left: Strip blots of yeast protein extracts stained with the indicated EnCor antibodies; MCA-38F3 is first lane on the left and stains a single band at ~34 kDa. **Right:** Human SH-SY5Y cells stained with MCA-38F3, showing prominent specular nucleolar staining. The nuclei are counter stained with blue DAPI DNA stain, so these spots appear very pale blue. The cells are also stained with EnCor's chicken antibody to neurofilament NF-H [CPCA-NF-H](#).

Diagram of Domain Structure: Generated from sequence of yeast Nop1p with [SMART](#) program from EMBL in Heidelberg. PFAM: fibrillarin refers to the fibrillarin domain. Scale is number of amino acids;



Antibody Characteristics: To raise the MCA-38F3 antibody, mice were injected with yeast nuclear preparations and hybridomas were screened by immunofluorescence on yeast cells and by western blotting on yeast protein homogenates. Reference 2 below describes the characterization of D77, an antibody very similar but not identical to MCA-38F3. This clone was selected because it stains a single ~34kDa band on western blotting and shows a clear and strong punctate staining of yeast nuclei. Subsequently it was found that this antibody recognizes fibrillarin in a wide variety of species including human, rat, *Drosophila*, *S. pombe*, *C. elegans*, and plants. It can therefore be used to identify nucleoli immunocytochemically. We supply this antibody as sterile-filtered cell culture fluid from an Integra CL-350 biochamber plus sodium azide. We currently produce 500 µL aliquots of this material. The immunoglobulin subtype is IgG1. The exact concentration of IgG is unknown.

Suggestions for Use: For western blots of yeast protein samples, use MCA-38F3 diluted 1/2,000 (cell lysates) to 1/10,000 (nuclear fractions), followed by chemiluminescent detection (ECL). For other (non-ECL) western detection methods, try MCA-38F3 diluted 1/1,000 to 1/5,000. To detect mammalian fibrillarin on western blots by ECL, try MCA-38F3 at 1/500 dilution. For immunofluorescence on yeast cells, use MCA-38F3 diluted 1/1,000 to 1/5,000. For IF of mammalian cells, try MCA-38F3 at 1/500. Antibody preparation contains 10mM sodium azide preservative. Avoid repeated freezing and thawing, store at 4°C or -20°C.

References:

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3. 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 \(2003\)](#).
4. Tyagi S and Alsmadi O. Imaging native beta-actin mRNA in motile fibroblasts. [Biophys J. 87:4153-62 \(2004\)](#).
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6. Vermaak D, Henikoff S, Malik HS. Positive selection drives the evolution of rhino, a member of the heterochromatin protein 1 family in *Drosophila*. [PLoS Genetics 1:96-108 \(2005\)](#).

Limitations: This product is for research use only and is not approved for use in humans or in clinical diagnosis.

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