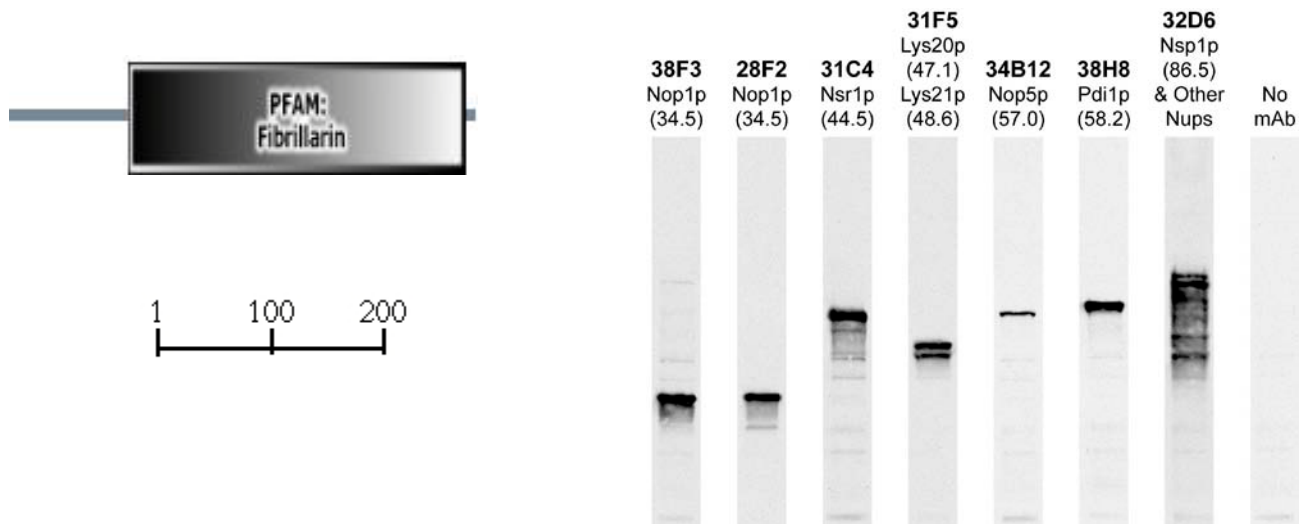


**Catalogue # MCA-28F2: Monoclonal Antibody 28F2**

**The Immunogen:** Nop1p was originally identified as a nucleolar protein of bakers yeast, *Saccharomyces cerevisiae* (accession P15646). The Nop1p protein is essential for yeast viability and is localized in the nucleoli. The human homologue of Nop1p is fibrillarin (accession P22087) a component of the nucleolar small nuclear ribonucleoprotein (snRNP) particle. The human fibrillarin gene is located on chromosome 19 (19q13.1). Fibrillarin proteins have been cloned and sequenced from several other species (Mouse, accession P35550, *Xenopus* accession P22232, *C. elegans* accession Q22053, and *S. pombe* accession P35551). You can download a pdf of the sequence alignment here. The N terminal ~80 amino acids contain multiple copies based on the peptide RGG, and the remaining ~240 amino acids consist of the fibrillarin domain. A fibrillarin homologue has also been identified in the genome of the archean *Methanococcus* (accession NC\_000909). This protein lacks the RGG rich N-terminal extension but is clearly homologues to the other sequences throughout all of the fibrillarin domain. The structure of this molecule has been determined and shown to consist of 2 extended b-sheets flanked by 4 a-helices. Patients with the autoimmune disease scleroderma often have circulating autoantibodies to a 34 kDa protein which was subsequently found to be fibrillarin. To raise this 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. This monoclonal antibody does **not** cross-react with fibrillarin in other species, but is an excellent marker of the nucleolus of yeast cells. For Nop1p/fibrillarin visualization in vertebrates, *Drosophila* and *C. elegans* and other species we recommend MCA-38F3, also available from EnCor.



**Diagram of Domain Structure (above left):** 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 specificity (above right):** Western blots of whole yeast protein extracts with a collection of our antibodies. The blot for MCA-28F2 is in the indicated lane, and the number indicates the SDS-PAGE molecular weight in kiloDaltons.

**Antibody Characteristics:** Sterile-filtered cell culture fluid from an Integra CL-350 biochamber plus sodium azide. The immunoglobulin subtype is IgG3. The concentration of IgG is unknown. Store at 4°C or -20°C. For western blots of yeast protein samples, use MCA-28F2 diluted 1/10,000 (cell lysates) to 1/25,000 (nuclear fractions), followed by chemiluminescent detection (ECL). See here for image of blot. For other (non-ECL) western detection methods, try MCA-28F2 diluted 1/1000 to 1/5000. For immunofluorescence on yeast cells, use MCA-28F2 diluted

1/2000 to 1/20,000. We routinely dilute to 1/5000. For immunoprecipitations, use 2-4  $\mu$ L for an amount of lysate derived from 5 OD600 units of yeast culture.

**References:**

1. Ochs RL, Lischwe MA, Spohn WH, Busch H. Fibrillarin: a new protein of the nucleolus identified by autoimmune sera. [Biol Cell 54:123-133 \(1985\)](#).
2. 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](#).
3. Tsang CK, Bertram PG, Ai W, Drenan R, Zheng XF. Chromatin-mediated regulation of nucleolar structure and RNA Pol I localization by TOR. [EMBO J. 22:6045-56 \(2003\)](#).
4. Porter SE, Penheiter KL, Jaehning JA. Separation of the *Saccharomyces cerevisiae* Paf1 Complex from RNA Polymerase II Results in Changes in Its Subnuclear Localization. [Eukaryotic Cell 4:209-220 \(2004\)](#).
5. Hochwagen A, Tham W, Brar G, Amon, A. The FK506 Binding Protein Fpr3 Counteracts Protein Phosphatase 1 to Maintain Meiotic Recombination Checkpoint Activity [Cell 122:861-873 \(2005\)](#).

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

©EnCor Biotechnology Inc. June 23, 2014.