Monoclonal Antibodies for Yeast Cell Biology

Quick Facts

Storage upon receipt:

- –20°C
- Desiccate

Introduction

Molecular Probes offers a diverse selection of monoclonal antibodies (mAbs) that are useful for studying many aspects of cell biology with the yeast *Saccharomyces cerevisiae* (Table 1). Five of these antibodies specifically bind to proteins that reside in the yeast vacuole, two antibodies are specific for mitochondrial membrane proteins, one antibody is specific for an endoplasmic reticulum integral membrane protein, one antibody is specific for an endosomal membrane protein, one antibody is specific for a late Golgi membrane protein and one antibody is specific for a cytosolic enzyme.

The five anti-yeast vacuolar protein mAbs include two that are specific for peripheral membrane subunits of the vacuolar H^+-ATPase (V-ATPase). The mAb 13D11 recognizes the 60 kDa subunit [1] (the B-subunit, the product of the *VMA2* or *VAT2* gene), whereas mAb 8B1 binds the 69 kDa subunit [2] (the A-subunit, the product of the *VMA1* or *TFP1* gene). These two polypeptides are associated with the cytoplasmic face of the vacuolar membrane when the V-ATPase complex is fully assembled.[2] The mAb 10D7 recognizes the 100 kDa V-ATPase subunit [3] (the product of the *VPH1* gene),[4] which is an integral membrane protein residing in the vacuolar membrane.[3,4] Monoclonal 1D3 is specific for alkaline phosphatase (ALP),[5,6] the product of the *PHO8* gene and an abundant ~70 kDa integral vacuolar membrane protein. The fifth anti-yeast vacuolar protein mAb (10A5) is specific for

Table 1. *Monoclonal antibodies for yeast cell biology.*

Catalog Number	Yeast Antigen Recognized By Antibody	Yeast Organelle in Which Antigen Resides	Monoclonal Antibody Number	Antibody Isotype	Western Blots * (µg/mL)	Immunocyto-chemistry * (µg/mL)	Refs
A-6422	V-ATPase 69 kDa Subunit	Vacuole Membrane	8B1	$IgG_{2a,\kappa}$	0.25	20	2, 3, 18
A-6426	V-ATPase 100 kDa Subunit	Vacuole Membrane	10D7	$IgG_{2a,\kappa}$	0.25	20	3, 16, 18
A-6427	V-ATPase 60 kDa Subunit	Vacuole Membrane	13D11	$IgG_{1,\kappa}$	0.25	20 †	1, 3, 5, 16, 18
A-6458	Alkaline Phosphatase (ALP, ~70 kDa)	Vacuole Membrane	1D3	$IgG_{1,\kappa}$	‡	† §	5, 13
A-6428	Carboxypeptidase Y (CPY, 61 kDa)	Vacuole Lumen	10A5	$IgG_{1,\kappa}$	0.5	20	7, 13
A-6408	Cytochrome Oxidase Subunit III (26 kDa)	Mitochondrial Inner Membrane	DA5	$IgG_{2a,\kappa}$	0.5	5–20	11
A-6449	Mitochondrial Porin (~30 kDa)	Mitochondrial Outer Membrane	16G9	$IgG_{1,\kappa}$	0.5	5–20 †	9, 10
A-6429	Dol-P-Man Synthase (Dpm1p, ~30 kDa))	Endoplasmic Reticulum Membrane	5C5	$IgG_{1,\kappa}$	4.0	NA	12, 13
A-21273	Pep12p (~37 kDa)	Endosomal Membrane	2C3	$IgG_{1,\kappa}$	0.5	1	13–15
A-21274	Vps10p (~180 kDa)	Late Golgi Membrane	18C8	$IgG_{2a,\kappa}$	4.0	NA	13
A-6457	3-Phosphoglycerate Kinase (PGK, 45 kDa)	Cytoplasm	22C5	$IgG_{1,\kappa}$	2.0	10	10, 12, 13

* Suggested antibody concentration, in µg/mL, for Western blots or immunocytochemistry. † These monoclonal antibodies yield the strongest and most consistent signals when used for indirect immunofluorescence; the anti-ALP antibody is the most reliable monoclonal antibody for detecting yeast vacuolar membranes. ‡ 50–100-fold dilution of the provided solution. § two- to threefold dilution of the provided solution. NA, not applicable (not recommended).

carboxypeptidase Y (CPY),[7,8] which is a 61 kDa soluble glycoprotein located in the vacuolar lumen.

For the detection of mitochondrial membranes, the anti-yeast mitochondrial porin mAb 16G9 [9,10] and anti-cytochrome oxidase subunit III mAb DA5 [11] are available. Porin is an abundant ~30 kDa integral membrane protein that resides in the outer membrane of yeast mitochondria, and cytochrome oxidase subunit III (COX III) is a 26 kDa integral membrane protein that resides in the inner mitochondrial membrane.

For the detection of yeast endoplasmic reticulum membranes, we offer the anti-dolichol phosphate mannose synthase (Dol-P-Man synthase, Dpm1p) mAb 5C5.[12] The yeast Dol-P-Man synthase is a ~30 kDa integral membrane protein that resides in the endoplasmic reticulum, and the mAb was prepared against the cytosolic domain of the protein.[13]

For the detection of the yeast endosomal compartment, we offer anti-Pep12p, mAb 2C3.[14-15] Yeast Pep12p, also known as Vps6p, is a ~37 kDa yeast endosomal protein of the t-SNARE or syntaxin family. Pep12p is localized via a C-terminal transmembrane domain, and the epitope recognized by mAb 2C3 is located in the N-terminal cytosolic domain.[13]

For the detection of the late-Golgi compartment of yeast, we offer anti-Vps10p, mAb 18C8.[13] Yeast Vps10p is a ~180 kDa membrane protein that resides in the late-Golgi compartment.

For the detection of yeast cytosol, we offer the anti–3-phosphoglycerate kinase (PGK) mAb 22C5.[10,12,13] PGK (EC 2.7.2.3) is an abundant 44.7 kDa yeast cytosolic protein.

Materials

Most of the the monoclonal antibodies are supplied as lyophilized powder in unit sizes of 250 µg. Upon receipt, the lyophilized antibodies should be stored desiccated at -20°C. To prepare stock solutions, reconstitute these antibodies in 0.2–1.0 mL of phosphate-buffered saline (PBS), pH 7.4, containing 1% 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. Anti-Pep12p (A-21273) is provided in a unit size of 100 µL and anti-Vps10p (A-21274) in a unit size of 500 µL as 0.5 mg/mL solutions of purified antibody in PBS containing 5 mM sodium azide. Upon receipt, store at -20°C. Anti-ALP (A-6458) is provided unpurified in a unit size of 2.5 mL as conditioned cell culture medium containing 10% fetal bovine serum and 5 mM sodium azide. Upon receipt, store at -20°C. When properly stored, these products are stable for at least six months. AVOID REPEATED FREEZING AND THAWING.

Specifications

The anti–V-ATPase subunit mAbs were prepared against purified *S. cerevisiae* vacuole membranes, and the anti-CPY mAb was prepared against purified *S. cerevisiae* CPY.[13] The anti-porin, anti-COX III and anti-PGK mAbs were prepared against the corresponding proteins isolated from *S. cerevisiae*.[13] The anti–Dol-P-Man synthase mAb was prepared against Dol-P-Man synthase expressed in and purified from *Escherichia coli*.[13] The anti-ALP mAb was prepared against an ALP–glutathione *S*-transferase (ALP–GST) fusion protein;[5] the anti-Pep12p mAb, against a Pep12–GST fusion protein;[13] and the anti-Vps10p mAb, against a Vps10–maltose-binding protein fusion protein.[13]

The purity and yield of each preparation was assessed by SDS-polyacrylamide gel electrophoresis and quantitative immunoassay specific for mouse IgG of the appropriate isotype. The antibody binding specificity was determined by particle-concentration fluorescence immunoassay (PCFIA) and/or enzyme-linked immunosorbent assay (ELISA) using the purified native proteins, except for anti–Dol-P-Man synthase, anti-ALP, anti-Pep12p and anti-Vps10p, in which case the recombinant Dol-P-man synthase protein, the ALP–GST fusion protein, a Pep12–His6 fusion protein and a Vps10–maltose-binding protein fusion protein, respectively, were used. Antibody binding specificity was also analyzed by Western blot immunoassay using protein extracts from yeast cells.

In addition to ELISA and Western blot analysis, each mAb (except for anti–Dol-P-Man synthase) has been used to detect its corresponding antigen by indirect immunofluorescence microscopy of fixed yeast cells. For the detection of Vps10p by anti-Vps10p, a yeast strain overproducing Vps10p was required. Thus, a majority of the mAbs can be used to detect native (solid-phase ELISA-like binding assays), denatured (Western blot analysis) and fixed (indirect immunofluorescence assays) antigens. In addition, both the anti–V-ATPase and anti-COX III mAbs are capable of immunoprecipitating either the V-ATPase or cytochrome oxidase complexes from detergent-solubilized yeast cells. The specificity of each mAb, the immunoglobulin isotype and other details of the mAbs are listed in Table 1.

Application

Molecular Probes has selected this set of mAbs because they are useful for both Western blotting of denatured proteins and protein immunolocalization in fixed yeast cells, as well as for immunoprecipitation of either the V-ATPase or cytochrome oxidase complex. Other uses of these mAbs include the development of ELISA assays to determine either the level of enrichment of a particular yeast organelle or the level at which the organelle contaminates a membrane preparation.

Indirect Immunofluorescence

Yeast organelles can be detected in fixed cells by indirect immunofluorescence using most of mAbs described here. However, three mAbs of this set are particularly useful:

- For detecting *vacuolar membranes*, the anti–V-ATPase 60 kDa subunit, mAb 13D11 [1,3,5,16] and the anti-ALP mAb 1D3 are recommended.[5,6]

- For detecting *mitochondrial membranes*, the anti-porin mAb 16G9 is the antibody of choice.[9,10,13]

While the anti–V-ATPase 100 kDa subunit mAb has been used in indirect immunofluorescence studies,[3,16] the epitope that it recognizes is masked in wild-type cells and is only revealed when a substantial fraction of the V-ATPase complex does not assemble onto the yeast vacuolar membrane.[3] Anti-CPY antibodies have also been used for indirect immunofluorescence studies in fixed yeast cells;[1] however, antibodies specific for lumenal vacuolar proteins typically give inconsistent staining patterns of poor quality relative to the staining patterns exhibited by antibodies specific for vacuolar surface proteins.[13,17] Although anti–COX III mAb DA5 can be used to localize yeast mitochondria by immunofluorescence, more intense

mitochondrial staining can be obtained by using mAb 16G9 to reveal porin,[13] which is a more abundant mitochondrial protein. The anti–Dol-P-Man synthase and the anti-Vps10p mAbs are not recommended for immunocytochemistry, although Vps10p can be detected in cells that overproduce Vps10p.[13]

Subcellular Fractionation

All of the mAbs are useful for identifying organelles by Western blot analysis during subcellular fractionation of yeast membranes.

- For detecting the presence of *vacuolar membranes*, the anti–V-ATPase 100 kDa subunit mAb 10D7 and the anti-ALP mAb 1D3 are the antibodies of choice because the V-ATPase 100 kDa subunit and ALP are integral membrane proteins that are not stripped from membranes during fractionation.[3-5,7,18]

- For the detection of *mitochondrial membranes*, either the anti-porin mAb 16G9 or the anti-COX III mAb DA5 is appropriate.[11,13] Both porin and COX III are integral membrane proteins so they stay tightly associated with mitochondrial membranes during fractionation protocols.

- For the detection of *endoplasmic reticulum membranes* during subcellular fractionation of yeast membranes, the anti–Dol-P-Man synthase mAb 5C5 is highly useful.[12,13]

- For the detection of *endosomal membrane* fractions, the anti-Pep12p mAb 2C3 is recommended.[14,15]

- For the detection of *late-Golgi membrane* fractions, the anti-Vps10p mAb 18C8 is recommended.[13]

- For the detection of *cytosolic proteins*, we offer the anti-PGK mAb 22C5.[10,12,13] Because PGK is an abundant, fully soluble protein that does not sediment at 200,000 × g, its presence or absence will provide a critical test for cytosolic contamination of subcellular fractions.

Immunoprecipitation

Both the anti–V-ATPase 69 kDa subunit mAb 8B1 and the anti–V-ATPase 100 kDa subunit mAb 10D7 are extremely useful for immunoprecipitation of the fully assembled and enzymatically active V-ATPase complex.[2,18] Similarly, the anti-COX III mAb DA5 can be used to immunoprecipitate the fully assembled cytochrome oxidase enzyme complex.

Screening for CPY Secretion

The anti-CPY mAb 10A5 is ideal for screening for CPY secretion (Vps^- phenotype) in yeast using the colony immunoblot overlay assay or for detecting the level of CPY in yeast cells by Western blotting.[13] The anti-CPY mAb has been extensively used in screens for new *vps* mutations,[7,13,17] as well as in complementation analysis with the *vps* mutant collection.[13,17]

References

1. Mol Biol Cell 3, 1389 (1992); **2.** J Biol Chem 264, 19236 (1989); **3.** J Biol Chem 267, 447 (1992); **4.** J Biol Chem 267, 14294 (1992); **5.** Mol Cell Biol 16, 2700 (1996); **6.** J Cell Biol 136, 287 (1997); **7.** Eur J Cell Biol 65, 305 (1994); **8.** Genetics 144, 445 (1996); **9.** Mol Biol Cell 9, 917 (1998); **10.** J Cell Biol 143, 333 (1998); **11.** J Biol Chem 268, 18754 (1993); **12.** J Biol Chem 272, 25928 (1997); **13.** T.H. Stevens Laboratory, Institute of Molecular Biology, University of Oregon, unpublished; **14.** Traffic 1, 45 (2000); **15.** Mol Biol Cell 11, 305 (2000); **16.** Mol Biol Cell 7, 985 (1996); **17.** Methods Enzymol 194, 644 (1991); **18.** J Biol Chem 268, 16845 (1993).

Product List

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

Cat #	Product Name	Unit Size
A-6408	anti-OxPhos Complex IV subunit III (yeast), mouse monoclonal DA5 (anti-cytochrome oxidase subunit III (yeast)) *human reactivity*	250 µg
A-6429	anti-dolichol phosphate mannose synthase (yeast), mouse monoclonal 5C5	250 µg
A-6449	anti-porin (yeast mitochondrial), mouse monoclonal 16G9	250 µg
A-21273	anti-Pep12p (yeast), mouse monoclonal 2C3 *0.5 mg/mL*	100 µL
A-6457	anti-3-phosphoglycerate kinase (yeast), mouse monoclonal 22C5 (anti-PGK)	250 µg
A-6458	anti-alkaline phosphatase (yeast vacuolar), mouse monoclonal 1D3 *in conditioned culture medium*	2.5 mL
A-6428	anti-carboxypeptidase Y (yeast vacuolar), mouse monoclonal 10A5 (anti-CPY)	250 µg
A-6427	anti-H^+-ATPase 60 kDa subunit (yeast vacuolar), mouse monoclonal 13D11	250 µg
A-6422	anti-H^+-ATPase 69 kDa subunit (yeast vacuolar), mouse monoclonal 8B1	250 µg
A-6426	anti-H^+-ATPase 100 kDa subunit (yeast vacuolar), mouse monoclonal 10D7	250 µg
A-21274	anti-Vps10p (yeast), mouse monoclonal 18C8 *0.5 mg/mL*	500 µL

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