Revised: 14-July-2003

Monoclonal Antibodies Specific for Yeast Cytochrome Oxidase Subunits

Quick Facts

Storage upon receipt:
- ≤–20°C
- Desiccate

Introduction

Cytochrome oxidase (COX) catalyzes the transfer of electrons from reduced cytochrome *c* to molecular oxygen, with a concomitant translocation of protons across the mitochondrial inner membrane.[1] This mitochondrial membrane–bound enzyme is composed of both mitochondrial-encoded subunits (subunits I, II and III) and nuclear-encoded subunits (all others), with a total of 11 subunits for yeast COX. To facilitate the study of mitochondrial biogenesis and cytochrome oxidase structure, Molecular Probes offers subunit-specific anti–yeast COX monoclonal antibodies (see Table 1). The binding specificity exhibited by these antibody preparations allows researchers to investigate the regulation, assembly and orientation of specific COX subunits.[2,3]

Contents and Storage

The anti–yeast COX antibodies are supplied in unit sizes of either 250 µg or 100 µg. Upon receipt, the lyophilized antibodies should be stored desiccated at ≤–20°C. When properly stored, these products are stable for at least one year.

To prepare stock solutions, reconstitute these antibodies in 0.5–1.0 mL of phosphate-buffered saline (PBS), pH 7.4, containing 1% BSA. Store the solutions for up to two months at 2–6°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.

Specifications

The anti–yeast COX monoclonal antibodies were prepared against COX isolated from *Saccharomyces cerevisiae*. The purity and yield of each preparation was assessed with 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 Western blot immunoassay. The subunit specificity and immunoglobulin isotype are shown in Table 1.

Application

The anti–yeast COX monoclonal antibody preparations will detect intact native yeast COX by solid-phase binding assays, such as PCFIA and enzyme-linked immunosorbent assay (ELISA). When bound to protein A–Sepharose® CL-4B, the antibody will immunoprecipitate the native yeast COX enzyme complex. In addition, each of these monoclonal antibodies recognizes the corresponding denatured COX subunit by Western blot immunoassay (Table 1) and may be used to test other subcellular preparations for mitochondrial contamination. Anti–yeast COX subunit III monoclonal DA5 can also be used to visualize mitochondria in fixed yeast cells using standard immunocytochemical techniques.

Table 1. *Anti–yeast COX monoclonal antibody specifications.*

Cat #	Mouse Monoclonal	COX Subunit Specificity	Subunit ** Molecular Weight	Isotype	Suggested Conc. for Westerns
A-6405	11D8-B7	Subunit I	56 kD	$IgG_{2b,\kappa}$	0.5 µg/mL
A-6407	4B12-A5	Subunit II	26.7 kD	$IgG_{2a,\kappa}$	1 µg/mL
A-6408	DA5	Subunit III *	30.3 kD	$IgG_{2a,\kappa}$	0.5 µg/mL
A-6432	1A12-A12	Subunit IV	14.9 kD	$IgG_{1,\kappa}$	0.5 µg/mL

* Monoclonal DA5 has also been shown to crossreact with *Neurospora crassa* subunit III. ** As determined by SDS-PAGE[4].

References

1. Annu Rev Biochem 59, 569 (1990); **2.** J Biol Chem 268, 18754 (1993); **3.** J Biol Chem 266, 7688 (1991); **4.** J Bioener Biomemb 20, 291 (1988).

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

Cat #	Product Name	Unit Size
A-6405	anti-yeast cytochrome oxidase subunit I, mouse monoclonal 11D8-B7	100 µg
A-6407	anti-yeast cytochrome oxidase subunit II, mouse monoclonal 4B12-A5	250 µg
A-6408	anti-yeast cytochrome oxidase subunit III, mouse monoclonal DA5	250 µg
A-6432	anti-yeast cytochrome oxidase subunit IV, mouse monoclonal 1A12-A12	250 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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