

PRODUCTS FOR RESEARCH INTO THE FUNCTION AND DYSFUNCTION OF MITOCHONDRIAL COMPLEX V

MS501 COMPLEX V IMMUNOCAPTURE KIT

Isolates Complex V, the F_1F_0 ATP synthase, from human, mouse and bovine tissues and cell lines

RESEARCH USES

The Complex V immunocapture kit allows isolation of the ATP synthase complex (E.C. 3.6.3.14) from small amounts of tissue. This facilitates subsequent analysis of assembly state and activity. Thus the enzyme retains oligomycin sensitive ATP hydrolysis activities after isolation. Finally, the extent of post translational modifications including oxidative damage can be readily analyzed by proteomic approaches or antibody detection of these modifications. Potential uses for the Complex V immunocapture kit include but are not limited to examining alterations of Complex V subunits in inherited mitochondrial diseases (1-4), Alzheimer's disease (5), angiogenesis (6,7), hypertension (8,9) and aging (10).

DESCRIPTION

The key component of the Complex V immunocapture kit is a monoclonal antibody able to selectively immunocapture the enzyme complex. The mAb is already covalently cross linked to Protein G-Agarose for convenience of use. This material is provided in batches of 65, 125 and 195 μ l beads which have been charged with approximately 250, 500 and 750 μ g of antibody respectively. When used as described in the protocol in Figure 1, 10 μ l of beads are able to immunocapture approximately 20 μ g of Complex V from heart mitochondria. Also provided are 2 mg of bovine heart mitochondria as a positive control to be used prior to, or during, isolation of Complex V from experimental samples. As an alternative, researchers can purchase the individual components i.e. 100 μ g of mAb and 2 mg BHM (kit MS501c).

SUGGESTED PROTOCOL FOR IMMUNOCAPTURING COMPLEX IV

The amount of Complex V that is captured in any experiment depends on both the amount of capture antibody and the amount of cell extract or isolated mitochondria used. Calculation of the amount of beads to be used in any experiment must also take account of the source of the material from which Complex V is to be isolated because mitochondria from different tissues have different concentrations of the enzyme complex. For example the levels of Complex V in mitochondria from cell culture material are around 10 fold less than in heart mitochondria. Figure 1 shows a schematic of a generic protocol developed for isolating Complex V from heart tissue. Figure 2 shows the levels of Complex V immunocaptured from different amounts of beef heart and human mitochondria by Coomassie blue

stained SDS-PAGE of immunoprecipitates obtained. Also shown is the identification of the subunits of immunocaptured Complex V by mass spectrometry from human heart mitochondria (11).

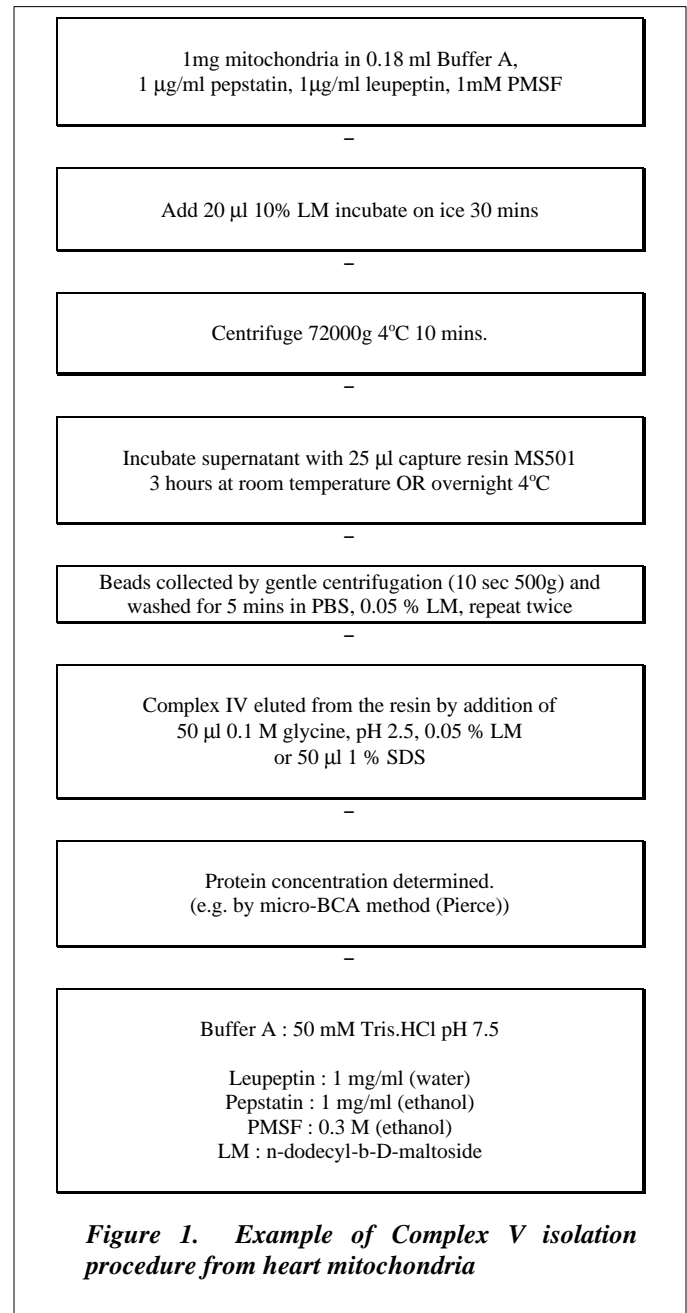
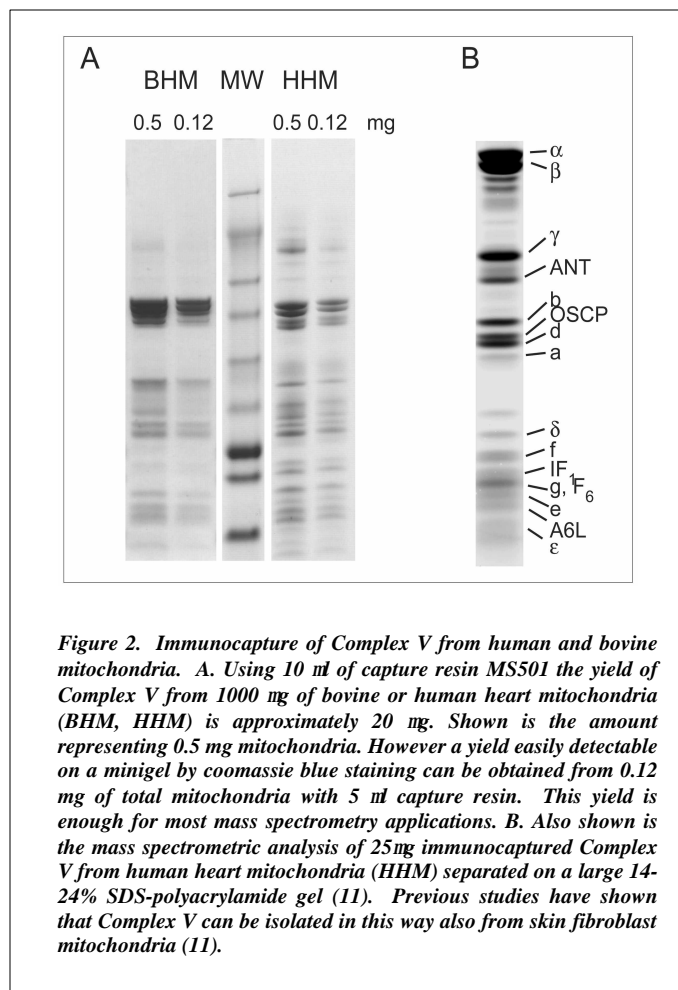


Figure 1. Example of Complex V isolation procedure from heart mitochondria

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140 mM NaCl, 2.7 mM KCl, pH 7.3) with 0.02 % sodium azide. Also included is 2 mg of purified bovine heart mitochondria resuspended in 100 µl of heart mitochondrial resuspension buffer (10 mM Tris HCl pH 7.8, 0.2 M sucrose, 0.2 mM EDTA, 1 mM PMSF). The antibody is shipped on ice. Upon receipt store the mAb at 4°C. The mitochondrial preparation should be aliquoted and stored at -20°C until use.

1. *Science* (1999) 283, 1482-1488
2. *Am J Med Genet* (2001) 106, 18-26
3. *J Clin Invest* (2003) 111, 303-312
4. *Nat Genet* (2002) 30, 394-399
5. *Neuroscience* (2003) 117, 293-303
6. *Proc Natl Acad Sci U S A* (1999) 96, 2811-2816
7. *Proc Natl Acad Sci U S A* (2001) 98, 6656-6661
8. *J Biol Chem* (1998) 273, 31778-31783
9. *J Clin Invest* (2001) 108, 1023-1030
10. *Biosci Rep* (1997) 17, 115-146
11. *J Biol Chem* (2002) 277, 33906-33912

Price List

All MitoSciences products are sold “FOR RESEARCH PURPOSES ONLY”

MATERIALS AND STORAGE

Kit MS501 contains the anti-Complex V immunocapture mAb covalently linked to protein G-Agarose beads in 65, 125 or 195 µl amounts. Approximately 4 µg of antibody is attached per 1 µl beads. All volumes of bead resin are suspended in 400 µl of PBS buffer (4.3 mM KH₂PO₄, 1.4 mM Na₂HPO₄,