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

MS401 COMPLEX IV IMMUNOCAPTURE KIT

Isolates cytochrome *c* oxidase, COX, from human and bovine tissues and cell lines

RESEARCH USES

The Complex IV immunocapture kit allows isolation of the cytochrome *c* oxidase complex (E.C. 1.9.3.1) from small amounts of tissue. This facilitates subsequent analysis of both assembly state and accrued post-translational modifications of all 13 subunits of the enzyme. The immunoprecipitated Complex IV shows cytochrome *c* oxidase activity while bound to the beads and this activity is fully sensitive to cyanide. Uses for the Complex IV immunocapture kit include but are not limited to examining alterations of Complex IV subunits in inherited mitochondrial diseases (1), Alzheimer's disease (2,3), schizophrenia (4) and cancer (5).

DESCRIPTION

The key component of the Complex IV 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 25, 50 and 75 µl beads which have been charged with approximately 250, 500 and 750 µg of antibody respectively. When used as described in the protocol in Figure 1, 10 µl of beads are able to immunocapture approximately 10 µg of Complex IV 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 IV from experimental samples. As an alternative, researchers can purchase the individual components i.e. 100 µg of mAb and 2 mg BHM (kit MS401c).

SUGGESTED PROTOCOL FOR IMMUNOCAPTURING COMPLEX IV

The amount of Complex IV 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 IV is to be isolated because mitochondria from different tissues have different concentrations of the enzyme complex. For example the levels of Complex IV in mitochondria from cell culture material are around 10 fold less that in heart mitochondria. Figure 1 shows a schematic of a generic protocol developed for isolating Complex IV from heart tissue.

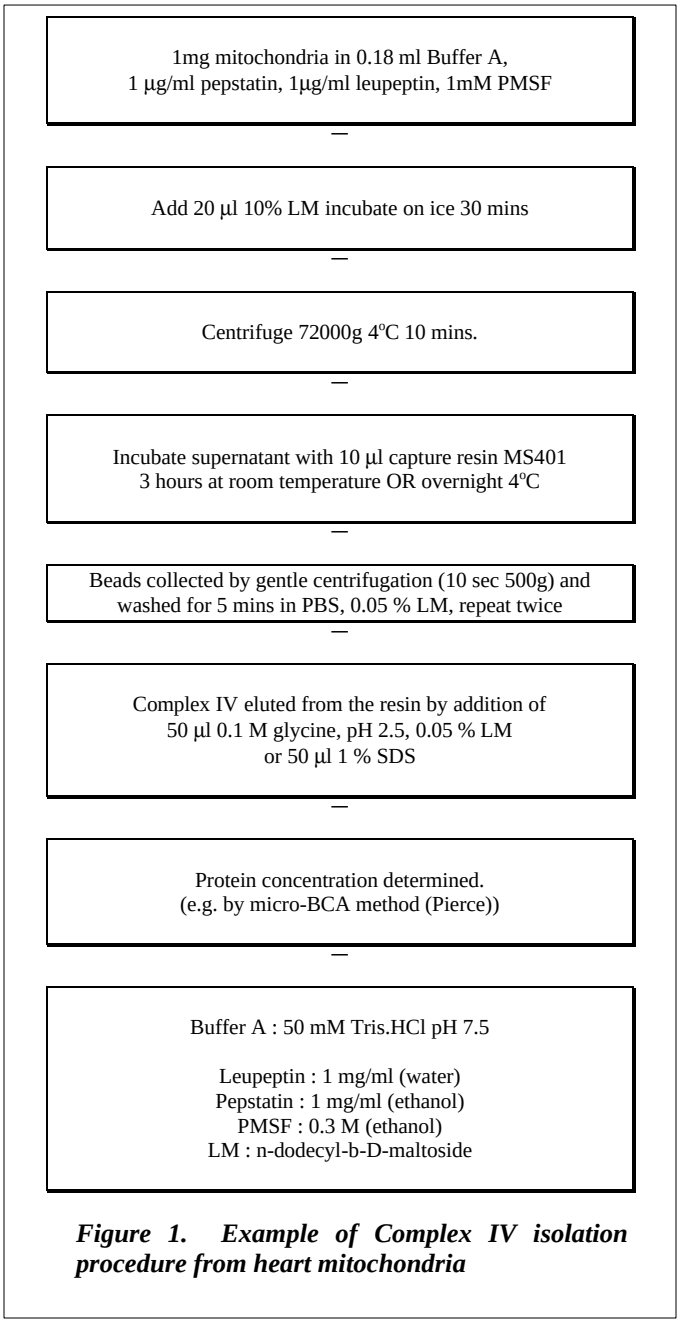

Figure 1. Example of Complex IV isolation procedure from heart mitochondria

MATERIALS AND STORAGE

Kit MS401 contains the anti-Complex IV immunocapture mAb covalently linked to protein G-Agarose beads in 25, 50 or 75 µl amounts. All volumes of bead resin are suspended in 400 µl of PBS buffer (1.4 mM KH_2PO_4, 8 mM Na_2HPO_4, 140 mM NaCl, 2.7 mM KCl, pH 7.3) with 0.02 % sodium azide. Also included are 2 mg of purified bovine heart

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mitochondria resuspended in 400 µl of heart mitochondria 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 for future use before storage at -20°C until use.

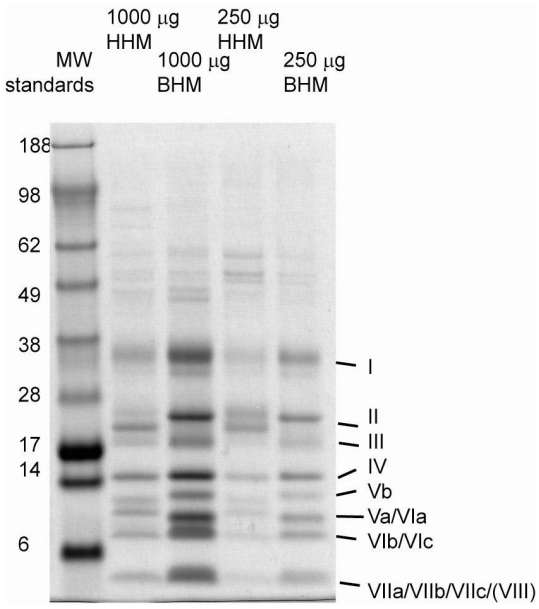

Figure 2. Immunocapture of Complex IV from human and bovine heart mitochondria. Using 10 ml of capture resin MS401 the yield of Complex IV from 1000 mg of bovine heart mitochondria (BHM) is approximately 10 mg. However a yield detectable by Coomassie staining can be obtained from less than 250 mg of total mitochondria. This yield is more than sufficient for mass spectrometry applications. Also shown is capture of Complex IV from human heart mitochondria (HHM). Complex IV can also be isolated in tissue specific forms form kidney, and liver as well as from brain mitochondria and cultured cells using this kit. Moreover extensive LC/MS/MS has been performed which has identified 12 of 13 Complex IV proteins. Only subunit VIII was not identified by mass

1. ***Neuropediatrics*** **(2003) 34, 311-317**
2. ***Exp Neurol*** **(2003) 182, 421-426**
3. ***Neurobiol Aging*** **(2000) 21, 455-462**
4. ***Schizophr Res*** **(2001) 48, 125-136**
5. ***Proteomics*** **(2003) 3, 1801-1810**