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

MS101 COMPLEX I IMMUNOCAPTURE KIT

Isolates NADH ubiquinone oxidoreductase complex, from human, rat, mouse and bovine tissues and cell lines

RESEARCH USES

The Complex I immunocapture kit allows isolation of the NADH ubiquinone oxidoreductase complex (E.C. 1.6.5.3) from small amounts of tissue. This facilitates subsequent analysis of assembly state and activity. Thus the enzyme retains NADH-ferricyanide, NADH-CoQ1 and NADH hexaaminoruthenium reductase activities after isolation, and with added lipids it also shows significant rotenone sensitivity. Finally, the extent of post translational modifications including oxidative damage can be readily analyzed by proteomic approaches or antibody detection of these modifications. Uses for the Complex I immunocapture kit include but are not limited to examining alterations of Complex I subunits in inherited mitochondrial diseases (1), Parkinson's disease (2), Alzheimer's disease (3), ALS (4) schizophrenia (5) and aging (6).

DESCRIPTION

The key component of the Complex I 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 25 µg of Complex I 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 I from experimental samples. As an alternative, researchers can purchase the individual components i.e. 100 µg of mAb and 2 mg BHM (kit MS101c).

SUGGESTED PROTOCOL FOR IMMUNOCAPTURING COMPLEX I

The amount of Complex I 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 source of the material from which Complex I is to be isolated because mitochondria from different tissues have different concentrations of the enzyme complex. For example the levels of Complex I 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 I from heart tissue.

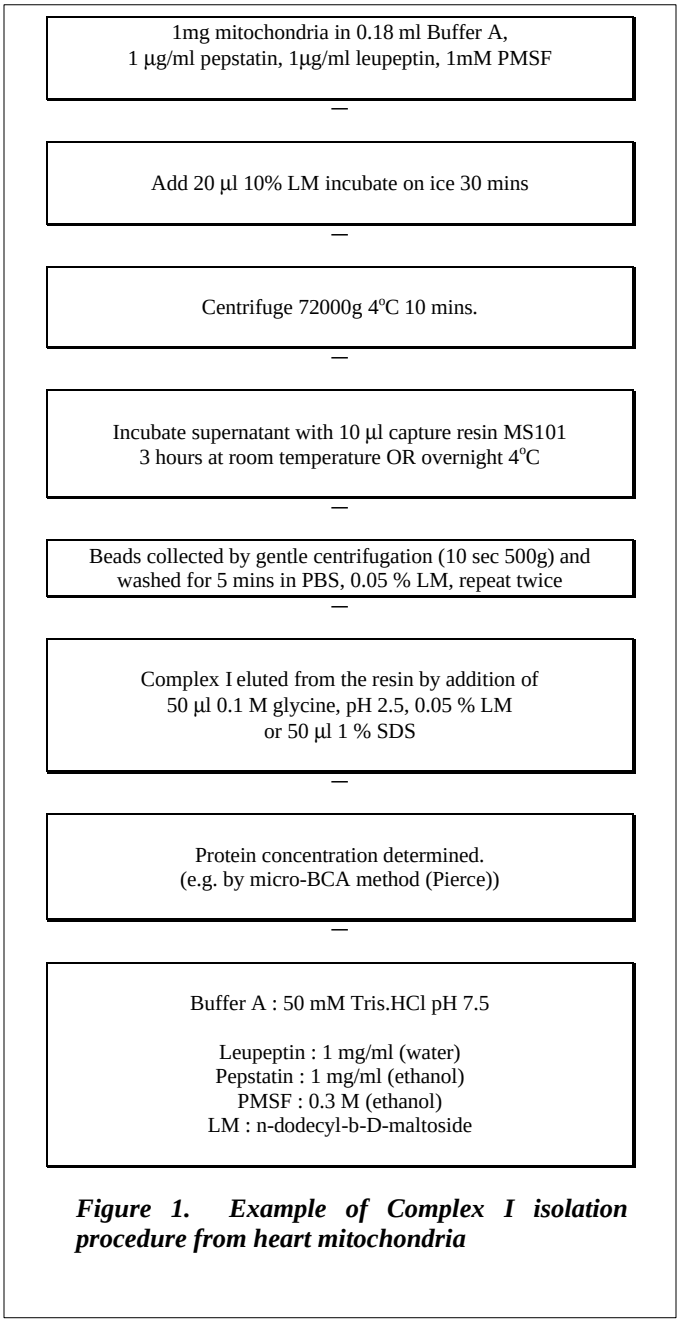

Figure 1. Example of Complex I isolation procedure from heart mitochondria

Figure 2 shows the levels of Complex I immunocaptured from different amounts of beef heart and human mitochondria by Coomassie blue stained SDS-PAGE of the immunoprecipitates obtained. Also shown are the amounts of Complex I that can be immunocaptured from 100 µg mouse heart mitochondria and 600 µg mouse brain mitochondria, respectively.

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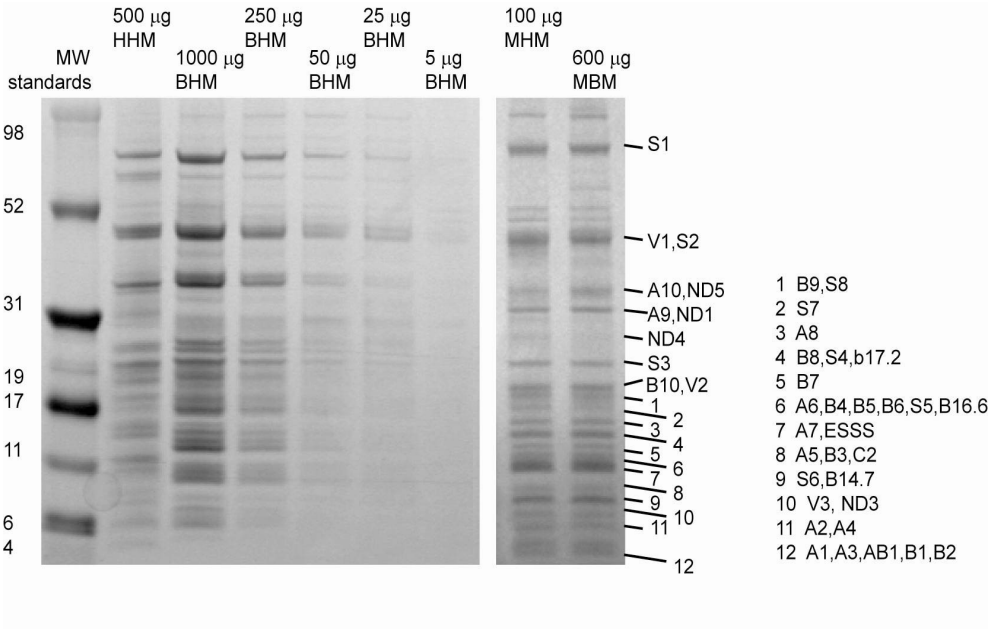

Figure 1. Immunocapture of Complex I from human, bovine and mouse mitochondria. Using 10 ml of capture resin MS101, the yield of Complex I from 1000 mg of bovine heart mitochondria (BHM) is approximately 25 mg. However a yield detectable by Coomassie staining can be obtained from as little as 25 mg of total mitochondria. This yield is enough for most mass spectrometry applications. Also shown is capture of Complex I from human heart mitochondria (HHM) and mouse heart mitochondria (MHM). Complex I can be isolated in this way from kidney, liver and brain mitochondria. Complex I from mouse brain mitochondria (MBM) has been subjected to extensive LC/MS/MS and 41 of 45 Complex I proteins identified in this way.

MATERIALS AND STORAGE

Kit MS101 contains the anti-Complex I immunocapture mAb covalently linked to protein G-Agarose beads in 25, 50 or 75 µl amounts. The beads have 8-10 µg mAb bound per µl bead volume. 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 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 and stored at -20°C until use.

1. ***Am J Med Genet (2001) 106, 37-45***
2. ***Bioessays (2002) 24, 308-318***
3. ***Life Sci (2001) 68, 2741-2750***
4. ***J Neurol Sci (1999) 169, 133-139***
5. ***Mol Psychiatry (2002) 7, 995-1001***
6. ***Biochim Biophys Acta (2000) 1459, 397-404***

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