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

MS301 COMPLEX III IMMUNOCAPTURE KIT

Isolates Complex III from human, mouse, rat and bovine tissues and cell lines

RESEARCH USES

The Complex III immunocapture kit allows isolation of the ubiquinol-cytochrome c reductase complex (E.C. 1.10.2.2) from small amounts of tissue. This facilitates subsequent analysis of assembly state, activity and the extent of post translational modifications including oxidative damage that occur with aging. Uses for the Complex III immunocapture kit include research on genetic mitochondrial disease (1), where complex III may have an additional role in the stabilization of complex I (2).

DESCRIPTION

The key component of the Complex III 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 75 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 III 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 III from experimental samples. As an alternative, researchers can purchase the individual components i.e. 100 µg of mAb and 2 mg BHM (kit MS301c).

SUGGESTED PROTOCOL FOR IMMUNOCAPTURING COMPLEX III

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

Figure 2 shows the subunit structure of Complex III immunocaptured from beef heart and human heart mitochondria by Coomassie blue stained SDS-PAGE of immunoprecipitates obtained. The subunits of the complex are clearly resolved.

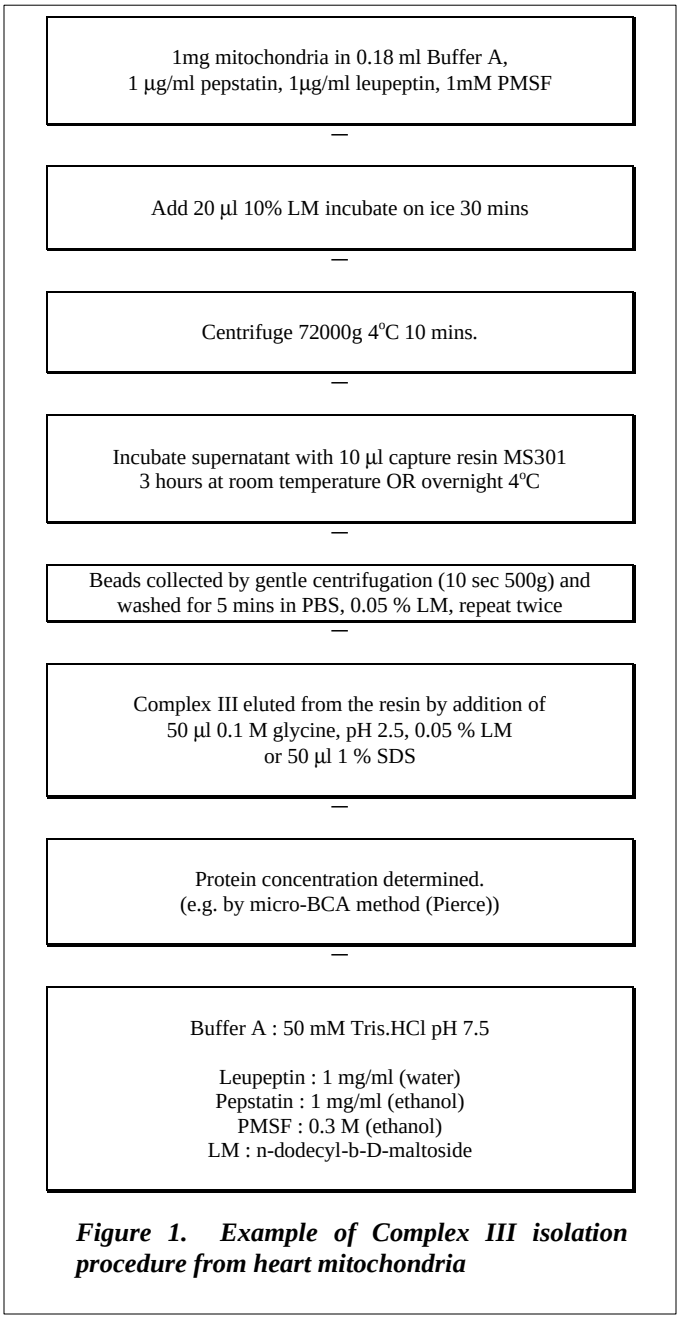

Figure 1. Example of Complex III isolation procedure from heart mitochondria

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

MATERIALS AND STORAGE

Kit MS301 contains the anti-Complex III immunocapture mAb covalently linked to protein G-agarose beads in 25, 50 or 75 µl amounts. 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.

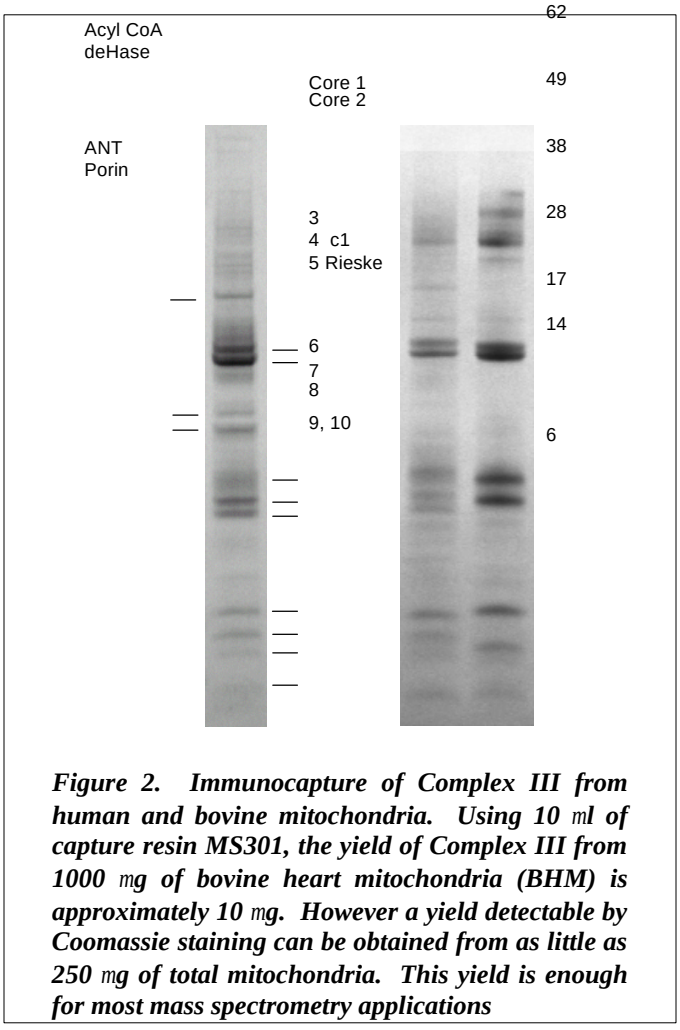

Figure 2. Immunocapture of Complex III from human and bovine mitochondria. Using 10 ml of capture resin MS301, the yield of Complex III from 1000 mg of bovine heart mitochondria (BHM) is approximately 10 mg. However a yield detectable by Coomassie staining can be obtained from as little as 250 mg of total mitochondria. This yield is enough for most mass spectrometry applications

1. ***J Clin Invest (2003) 111, 303-312***
2. ***Mol Cell (2004) 13, 805-815***

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