PRODUCTS FOR RESEARCH INTO THE PERMEABILITY TRANSITION PORE AND APOPTOTIC SIGNALLING

The important role that mitochondria play in apoptosis, also called programmed cell death, is no longer questioned (1-3). In response to intracellular signaling and a resulting redistribution of the bax and bcl2 classes of proteins to the organelle, mitochondria release cytochrome c and other intermembrane space proteins into the cytosol. Cytochrome c then reacts with APAF1 to initiate a chain of events that leads to the dismantling of the cell from within. Release of cytochrome c is thought to be linked directly to the functioning of the channel generated by a set of proteins with diverse functions that assemble with the apoptotic stimulus into the so called permeability transition pore (PTP) (4,5). Among the proteins constituting this pore are VDAC, also called porin, the adenine nucleotide translocase (ANT), cyclophilin D (NP_005720), and possibly hexokinase and the peripheral benzodiazepine receptor. Mitosciences provides mAbs to many of these PTP proteins and other apoptotic factors.

MSA01 ANT IMMUNOCAPTURE KIT

Isolates mitochondrial ANT from human, and bovine tissues and cell lines

DESCRIPTION

The key component of the ANT immunocapture kit is a monoclonal antibody able to selectively immunocapture ANT. The mAb is already covalently cross linked to Protein G-Agarose for convenience of use. This material is provided in batches of 25 µl beads which have been charged with approximately 250 µg of antibody respectively. When used as described in the protocol in Figure 1, 10 µl of beads are able to immunocapture approximately 1 µg of ANT 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 ANT from experimental samples. As an alternative, researchers can purchase the individual components i.e. 100 µg of mAb, and 2 mg BHM (kit MSA01c).

SUGGESTED PROTOCOL FOR IMMUNOCAPTURING COMPLEX ANT

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

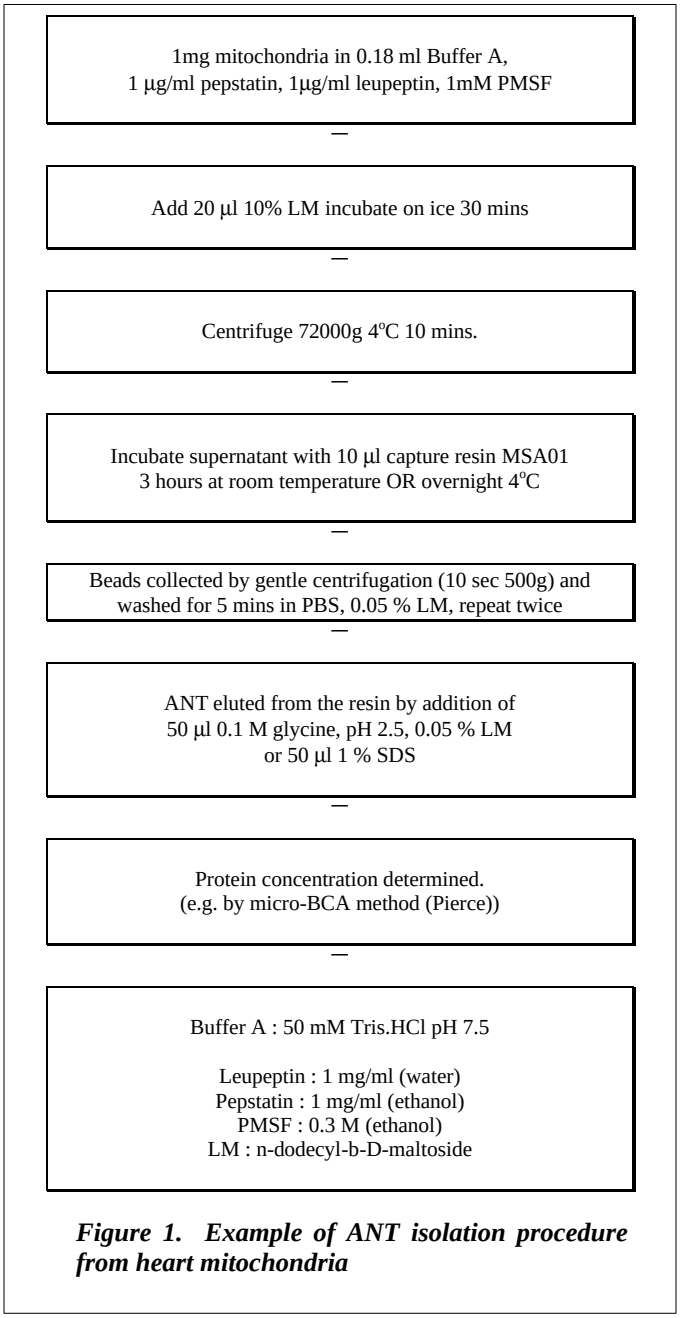

Figure 1. Example of ANT isolation procedure from heart mitochondria

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MATERIALS AND STORAGE

Kit MSA01 contains the anti-ANT immunocapture mAb covalently linked to protein G-agarose beads in 25 µl amounts. The beads have between 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 Anti-ANT mAb at 4°C. The mitochondrial preparation should be aliquoted and stored at -20°C until use.

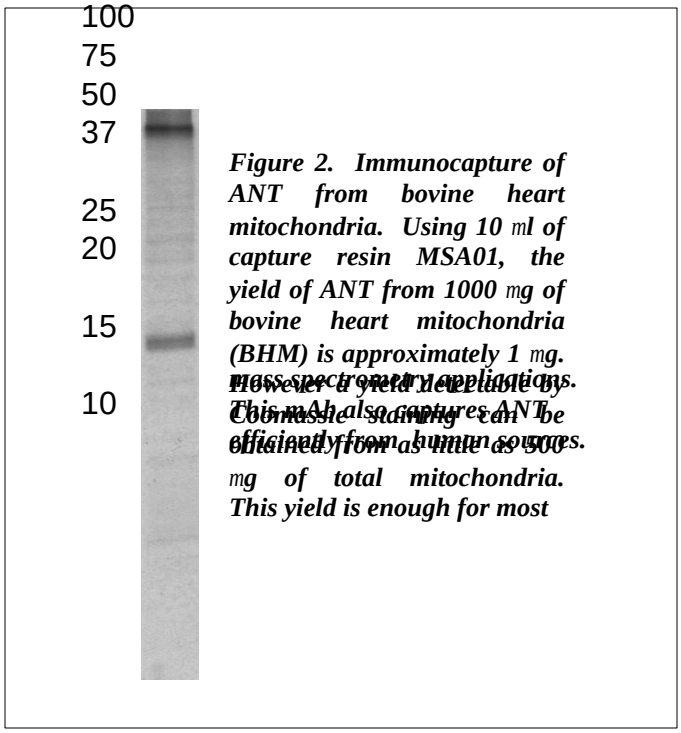

Figure 2. Immunocapture of ANT from bovine heart mitochondria. Using 10 ml of capture resin MSA01, the yield of ANT from 1000 mg of bovine heart mitochondria (BHM) is approximately 1 mg. However a yield detectable by Coomassie staining can be obtained from as little as 500 mg of total mitochondria. This yield is enough for most mass spectrometry applications. This mAb also captures ANT efficiently from human sources.

1. *Trends Genet (2002) 18, 142-149*
2. *Nat Med (2000) 6, 513-519*
3. *Trends Biochem Sci (2001) 26, 390-397*
4. *Faseb J (2002) 16, 607-609*
5. *FEBS Lett (1996) 397, 7-10*