

**S002** Study of protein complexes using Tandem Affinity Purification-Mass Spectrometry

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Most cellular processes are carried out by a multitude of proteins that assemble into multimeric complexes. A precise understanding of the biological pathways that control cellular events relies on the identification and on the biochemical characterization of the proteins involved in such multimeric assemblies. Advances in mass spectrometry (MS) have made possible the identification of multisubunit protein complexes isolated from cell lysates with high sensitivity and accuracy, whereas the tandem affinity purification (TAP) methodology efficiently isolates native protein complexes from cells for proteomics analyses. TAP is a generic method based on the sequential utilization of two affinity tags, to purify protein assemblies. During the first purification step, the protein A moiety of the TAP tag is bound to IgG beads, and protein components associated with the “TAP-tagged” protein are retrieved by tobacco etch virus (TEV) protease cleavage. This enzyme is a sequence-specific protease cleaving a 7-amino acid recognition site located between the first and second tags. In the second affinity step, the protein complex is immobilized to calmodulin coated beads via the calmodulin-binding peptide (CBP) of the TAP tag. The CBP-calmodulin interaction is calcium dependent and calcium chelating agents are used in the second elution step to release the final protein complex preparation used in MS analysis. The TAP-MS approach has proven to efficiently permit the characterization of protein complexes from bacteria, yeast, mammalian cells, as well as from multicellular organisms such as *C. elegans*, *Drosophila* and mice.