

MagneHis™ Ni-Particles

Markillie, L.M. *et al.* (2005) Simple protein complex purification and identification method for high-throughput mapping of protein interaction networks. *J. Proteome Res.* 4, 268–74.

To determine if the analysis of protein complexes could be streamlined, various affinity beads, elution and trypsin digestion conditions were optimized using the well-studied *Shewanella oneidensis* degradosome complex. The bait protein, polynucleotide phosphorylase (PNP, SO1209) from *S. oneidensis* MR-1, was cloned into a vector containing a polyhistidine affinity tag, expressed in *S. oneidensis* MR-1 cells and purified using Ni-NTA columns. Three different nickel-based magnetic beads, including MagneHis™ Ni-Particles, were evaluated. The bait protein was bound to the particles and resuspended at 1mg protein/ml beads, and the captured proteins were prepared from a cleared lysate of a *S. oneidensis* MR-1 culture. The immobilized bait protein was mixed with 10–20µg capture protein for 1 hour at 4°C, washed and eluted with denaturants. The eluted protein was digested with trypsin and analyzed by mass spectrometry.

The authors chose the MagneHis™ Ni-Particles for further optimization experiments, as the particles provided clean and reproducible pull-down results with minimal background. They developed a pull-down assay using MagneHis™ Ni-Particles followed by elution of protein complexes with acetonitrile, trypsin digestion in the elution buffer and analysis by mass spectrometry. This application of the MagneHis™ Ni-Particles minimizes protocol steps and is amenable to automated high-throughput analysis.

“...we compared the multiple nickel and cobalt beads listed in the Materials and Methods section for their binding capacity, nonspecific protein interaction, and pull-down efficiency (results not shown). The best matrix following those criteria appeared to be the MagneHis™ product from Promega.”

Markillie, L.M. *et al.* (2005) *J. Proteome Res.* 4, 268–74.

For additional protein purification citations, visit: www.promega.com/citations/

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Anti-β-Galactosidase mAb

Houghton, J. *et al.* (2004) Dual fluorescence experiment using the Anti-β-Galactosidase, Purified Monoclonal Antibody. *Science* 306, 1568–71.

Bone marrow-derived cells (BMDC) expressing bacterial β-galactosidase were transplanted into mice that were subsequently subjected to *Helicobacter felis* infection. After 52 weeks, gastric tumor tissue was harvested. Two antibodies, pan-cytokeratin indirectly labeled with fluorescein and anti-β-galactosidase mAb indirectly labeled with Cy[®]3, were used to identify BMDCs that had been recruited to the site of injury and differentiated into epithelial tissue.

For more details, see the eNotes article at: www.promega.com/enotes/applications/ap0072.htm

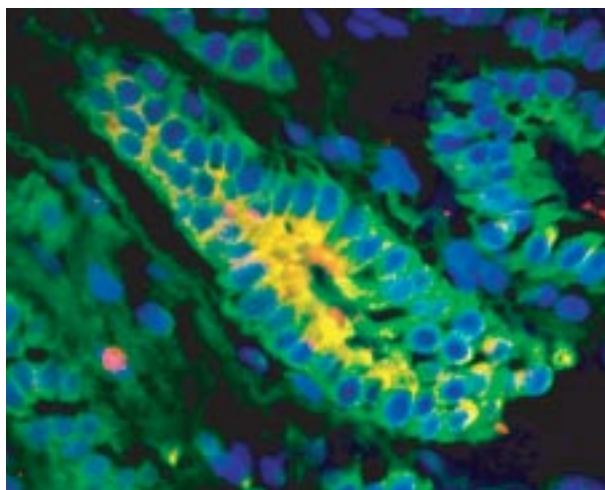


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