TECH NOTE

How can I decrease the amount of irrelevant protein captured with EXTRACTMAN®?

Because of its rapid, gentle, and minimally-dilutive extraction process, EXTRACTMAN® can recover a higher percentage of proteins that interact weakly and/or transiently with the paramagnetic particles (immunoprecipitation) or other captured proteins (co-immunoprecipitation).

While the washing process of the EXTRACTMAN® is often sufficient to remove background material that does not interact at all with the PMPs, it is not uncommon to recover higher concentrations of proteins that bind "non-specifically."

These non-specific proteins include proteins that are often "sticky" to the paramagnetic particles (PMPs), capture antibody/tag, or the target protein(s).

The presence of these proteins acts to convolute downstream analyses, particularly when using an analytical technique that interrogates all proteins in the eluent (e.g., silver stained SDS-PAGE, mass spectroscopy).

There are a number of different ways to reduce the quantity of these unwanted proteins:

1. "Pre-clear" the sample using PMPs with no antibody or tag—mix unlabeled PMPs with the sample for 10–30 minutes and then remove these PMPs in order to deplete proteins that non-specifically adhere to the PMP surface; once pre-cleared, processes the sample normally with EXTRACTMAN®.
2. Agitate the PMPs in one or more of the wash wells by sliding the release magnet of the EXTRACTMAN® back and forth several times beneath the PMPs, causing the PMPs to move up and down within the well; however, note that some loss of the target protein may also occur with this process.
3. Alternatively, drop the PMPs within an EXTRACTMAN® washing well and temporarily slide the EXTRACTMAN® handle to the side and briefly mix the PMPs using a micropipette; again, some loss of target product may occur.
4. Increase the stringency of the washing buffers.
5. Consider the use of alternative PMPs; we recommend Dynabeads® Protein G PMPs or Dynabeads M-270 PMPs.

   Dynabeads® is a registered trademark of Thermo Fisher Scientific, Inc.
6. Blocking of the beads with FBS for 20 minutes appears to reduce the background capture of some PMP types.
7. Be sure to run a control isolation that uses an irrelevant antibody and compare the proteins captured with the control (non-specific only) and the target antibody (non-specific and target) to identify target specific interactions.

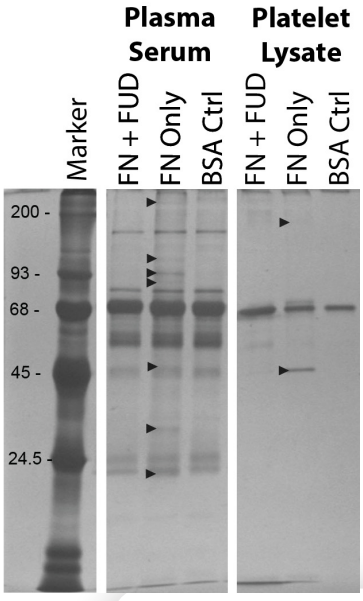

Figure – Using a prior version of the EXTRACTMAN® technology, proteins interacting with fibronectin (FN) were extracted from a human plasma sample and platelet lysate. As a control, PMPs bound with BSA (rather than fibronectin) were used. Both control and sample were analyzed via silver stained SDS-PAGE and differences between the gels were identified as proteins that specifically interacted with the fibronectin (marked with arrows). To demonstrate further specificity, a known interactor of fibronectin (FUD) was included in some trials to "block" these fibronectin specific interactions. Adapted from Moussavi-Harami et al., 2013.

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