H3/H4 depletion from crude extract using anti-H4K12ac antibodies

Use 2.4 µg anti-H4K12ac and 0.25 µl rPotein A sepharose (GE 17127901) per µl of extract to be depleted. For mock-depletion, use mouse IgG1 kappa Isotype Control (P3.6.2.8.1) from eBioscience

Note that these beads are a bit difficult to handle. We usually do washes in 0.5 ml protein lo-bind eppendorf tubes. In order to remove all the buffer and to not dilute the extract, we make small holes at the bottom of the tubes to spin out the wash buffers (see below for the washes). I strongly advise that before you try to couple antibody and deplete extract, you do a few test runs without antibody just to familiarize yourself with the method. Basically, there are three ways that things can go wrong. First, you can make holes that are too small. These might get clogged by the beads & buffer/extract won't spin out. Second, the holes can be too big & then you'll spin out both the buffer and the beads. Third, you can make mistakes when covering the bottom of the tube with parafilm & then you will lose antibody during the coupling reaction or lose extract during the depletion reaction.

For making holes, we use tweezers with a very fine tip (Dumostar 3c). We make a hole by using a single tip of the tweezers, that we push into the tube by about a millimeter. You'll need to play around with this a little bit before you get a feeling for what's good. We usually make three holes per tube. To cover the holes, we push two layers of parafilm onto the bottom of the tube. Make sure you "push" the parafilm on, rather than "pulling" it over the bottom of the tube, as the "pulling" can result in tears and subsequent leakage.

Take beads, wash twice in PBS pH7.4 (by spinning in one of these low-bind tubes with a small hole, 600g for 30sec), cover hole in the tube with parafilm, resuspend in appropriate amount of antibody (final concentration of antibody 1-2µg/µl in PBS pH7.4).
Rotate 1hr at room temperature. Save flow-through (quite a bit of the antibody won't actually bind to the beads, which is why so much antibody is needed. This is because this antibody unfortunately is of the IgG1 subtype that doesn't bind well to protein A or protein G. the flow through can be collected and concentrated & used again). Wash beads four times with 150µl PBS pH7.4 & once with sperm dilution buffer (this last wash spin 800 g for 1min so as not to dilute the extract with liquid trapped in the beads).

Add extract & rotate 38 min in the cold room. Spin out extract (600g, 30sec).

Treat extract with second round of uncoupled rProtein A sepharose beads (0.2 µl beads/µl extract), pre-washed once with PBS and once with SDB as above. Incubate with rotation in the cold room for 35min. This second beads treatment is to capture any antibody that may have leaked off the first beads. Recover extract by spinning as before. Use in experiments.

Note: I wouldn't deplete individual volumes larger than ~40 µl with this. If you want to deplete more than this, split into multiple tubes.

SDB: Sperm dilution buffer (5 mM HEPES, 100 mM KCl, 1 mM $MgCl_2$ and 150 mM sucrose, pH 8)