Pull down protocol:

Cell lysis first: use 1% Triton-X, 20mM Tris (pH7.5), 150mM NaCl, 1x protein inhibitor, 0.5mM MAEBSF, 0.5mM Na3VO4 and 10mM NaF. Add at least 2x the size of the pellet, incubate on ice for 30minutes, spin, and collect supernatant for pull down.

Wash beads:

* 2x Bead washing and binding buffer (as per manufactures instruction): 10mM Tris-HCl (pH7.5), 1mM EDTA and 2M NaCl.
* Resuspend dynabeads, transfer 25uL to tube per 10cm plate of cell lysate.
* Add equal amount of washing buffer and mix (vortex for 5sec)
* Add tube to magnet for 1min and discard supernatant.
* Repeat washing twice more.

Next step I’ve completed in two different ways with the same result thus far:

1. Preincubate lysate with biotinylated miRs.
   1. Add biotin-MiR (50pmol) to WCL (usually WCL volume is about 300uL)
   2. Incubate together for 1hr, on roller at room temp.
   3. Add 300uL of washing and binding buffer to WCL-Mir mix (protocol states that binding best occurs when NaCl levels decrease to 1M).
   4. Apply WCL-Mir-buffer to beads. Incubate for 2hr on roller.
   5. Add to magnet remove supernatant (I collect for comparison)
2. Incubate bio-mir to bead first then add lysate:
   1. Add biotin-MiR (50pmol) in 300uL washing and binding buffer to beads.
   2. Incubate for 30minute on roller.
   3. Add WCL to bead-mir mix. Incubate on roller for 3hrs.
   4. Add to magnet.

Washing non-binding material off: Add 100uL washing and binding buffer to beads, mix and then pellet (magnet), remove supernatant, repeat for a total of 4 washes.

Elute material: Add 0.1% SDS solution, incubate at 95 degrees for 10minutes, collect supernatant, and discard beads.

Variable elements:

* Salt and detergent concentration of the lysis buffer.
* Amount of bio-miR
* Amount of lysate/cells
* Cross-linking by formaldehyde.
* Type of beads: we have 4 different types (MyOne C1, MyOne T1, M280 and M270 streptavidin beads).
* Changing elution solution
* Changing washing/binding solution
* Changing to Immunoprecipitation using Anti-hnRNPK and pulling down RNAs.