Protocol for SpikeIP with Rutgers His-tagged peptide/domains.

Note:

a. This protocol is for testing 12 targets or 24 mAbs
b. Santacruz IgG (#SC-2025) was empirically determined to be 5times more concentrated than reported by the manufacturer. We therefore use a fifth of the needed amount to make it comparable to other antibodies in our experiment.

1. Prepare untransfected/naïve HEK or HeLa cell-pellets totaling to 10^8 cells.
2. Lyse the cells in 25ml of lysis buffer supplemented with protease inhibitors– resuspend cell pellet in buffer by vortexing. Incubate at RT for 10mins and spin at 4K/15mins. Use supernatant as whole cell-lysate.
3. Take out 30ul as 'unspiked control' for each gene being tested [360ul].
4. For each gene – we will perform 4 IPs
    a. IgG – (use 1ug/Rxn of Santacruz; #SC-2025)
    b. Anti- His (abcam – 1mg/ml – will use 5ul/Rxn)
    c. Anti- Gene-CDI mAB 1 (will use at 5ug/Rxn)
    d. Anti-Gene-CDI mAB 2 (will use at 5ug/Rxn; unless otherwise mentioned)
5. Each IP will be done using 500ul (i.e. ~$2x10^6$ cells) of whole-cell lysate with 1ug of peptide. Therefore in 2ml of lysate spike 4ug of peptide for each gene.
6. Take 30ul/spiked lysate for each gene as 'spiked input'
7. Incubate the spiked lysate with the respective antibodies overnight at 4C with rocking.
8. Next day incubate the lysate+antibody with 25ul proteinG dynabeads and incubate for 2hrs at 4C with rocking.
9. Spin down beads at 4k/2mins and discard supernatant.
10. Using a magnet base, wash the beads 3x with lysis buffer
11. Elute the beads in 30ul 1x LDS-sample loading buffer.
12. Run SDS-PAGE with unspiked lysate, spiked input and the four IP samples.
13. Visualize the blot using rabbit anti-His (Genescript; A00174-100).