Immunoprecipitation

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**All incubations to be done at 4°C on a 360° rotator
**Make sure to match the type of sepharose used to conjugate the antibody to the isotype of the antibody
***When piptetting sepharose beads, cut the pipette tips in half to enlarge the lumen of the tip to facilitate pipetting the beads (if you leave the tip uncut the tapered lumen of the tip will not allow you suck up the beads and you'll get more liquid then beads).

**Wash the sepharose with PBS prior to using as it is stored in an alcohol solution. Do this by pipetting whatever volume will be needed of sepharose for the experiment (volume depends on the amount of protein lysate in the experiment) and adding to an equal volume of PBS. Invert the tube several times. Spin 15,000 rpm x 2'. Then decant the sup and resuspend the beads with
an equal volume of PBS to the volume of beads after removal of the sup.

1) Pre-clear the tumor or cell lysates to remove nonspecific binding to the sepharose by incubating the lysates with sepharose (60 ul of sepharose per 1ml of lysate) for 1-2hrs at 4°C.
2) Spin 2-5' at 2,000 rpm at 4°C, then transfer the sup to a new tube
3) Add antibody to the sup at reccommended conc. for IP and incubate for at least 2hr or preferably ON.
4) Ppt the antibody-antigen complexes by adding the sepharose (40 ul, better to use less to avoid nonspecific protein binding) and incubate 1-2hr
5) Spin 5' at 1500 rpm, discard sup
6) Wash pellet with buffer used for the initial protein extraction but diluted 1:10 in PBS(this dilution is specific to the buffer used to extract Flk-1 at it contains Triton X-100 at too high a conc for the experiment, thus it must be diluted). Add 500 ul of wash solution to the sepharose pellet. Shake manually several times and spin 5' at 1500 rpm. Do this twice. The third wash is with straight 1XPBS. Spin down the beads after the last wash with PBS, discard the sup
7) Add Laemmli buffer to the sepharose pellets (add sufficient volume to run the samples on an acrylamide gel, usually 70ul per lane)
8) Boil at 100° for 5minutes to release the antigen from the antibody and from the sepharose beads
9) Spin for 5' at 15,000 rpm at RT to bring down the sepharose beads
10) Run the sup on an Acrylamide gel.