Criteria for passing individual mAbs:

The mAbs must pass primary (HuProt, OIRD or Octet) and secondary (WB or IP) levels of validation in order to be classified as a “passing reagent” for listing on this PCRP Data Portal.

For primary validation, HuProt, criteria for passing includes an A Score > 8 and an S Score > 3. For OIRD or Octet, Kd< 50 nM.

For secondary validation, the usefulness of antibodies in Immunoprecipitation (IP) and Immunoblotting (WB) applications were evaluated using preset and standardized thresholds for quality and efficiency. Listed below are lists of parameters that were considered when establishing the thresholds of these validations.

Immunoprecipitation (IP):

1. **Efficiency:** Following densitometric analysis of signal intensity in the immunoblots, test antibodies with greater than 0.15% IP-recovery, as compared to the total input sample, were considered as passers. Results from every antibody were manually inspected by at least two independent investigators before being passed.
2. **Protein Stability:** We observed target dependent variance in target stability during the transfection, storage and/or during the IP experimental procedure. As long as the target protein at the expected molecular weight was observed in the input and corresponding IP lanes, the reagent was considered to pass. The ability of the antibody to IP-recover the degraded fragments was not evaluated or considered towards the efficiency of the IP-recovery.
3. **Aberrant migration of the protein:** All constructs expressing the target ORF were verified for integrity by Sanger sequencing the construct expression cassette. However, we observed up to 20Kda super-shifts relative to the expected molecular weight for certain target proteins in our SDS-PAGE gels. As the protein was not visible in the lanes containing lysates of uninduced cells, we considered such cases to be under the normal purview of aberrant migration reported for many proteins in the literature.
4. **Signal in un-induced input lanes:** As we tested our IP experiments in Tet-ON HeLa or HEK cell lines, we expected to observe no expression of the transfected protein in the absence of tetracycline/doxycycline treatment. However, we occasionally observed leaky expression of the target protein even in the absence of doxycycline; a known caveat of the older-generation of tet-ON expression cassettes used in our cell-

lines. We considered the experiment to be acceptable if the levels of target protein visibly increased (at least 2-fold difference) when comparing the uninduced and highest induction samples in the input lanes.

5. **Non-specific signal in input lanes:** For unknown reasons, in a small subset of our experiments we observed that the rabbit anti-FLAG mAb (Cell-signaling) recognized non-specific signal(s) in all three input lanes. This signal didn't change in expression level as a function of doxycycline treatment and was not recovered by IP using the IgG or test antibodies. Such non-specific signal was not considered to be relevant towards the experimental evaluation of the antibody.
6. **Signal in IgG IP lanes:** We observed that certain target proteins had an increased propensity to form insoluble/sticky protein aggregates when expressed at high levels. This feature was readily visible as a strong signal in the IgG-IP lanes of our experiments. By serially titrating the level of induction by doxycycline, we were able to prevent this unwanted aggregation of target proteins with concomitant loss in signal intensity of the IgG-IP lanes. However, in certain scenarios a weak but visible signal might be present in in the IgG lane. If the densitometric signal observed in the IP lane (lane 6) using the test mAb was found to be at least 2x higher than the cognate signal in the IgG-IP lane (lane 4), we considered such an antibody to be a passing reagent.
7. **Failure of positive control IP:** In a significant fraction of our experiments we observed failure of recovery in our positive control using the mouse anti-FLAG IP (lane 7). As the FLAG tag is an N-terminal fusion to the target protein, a likely explanation for the absence of recovery is epitope masking/unavailability. Therefore, absence of a signal in the positive control was not considered to be a reason to fail the test antibody if all other results for that antibody fulfilled the passing criteria.

Immunoblotting (WB):

1. **Threshold:** Reagent was considered to pass if the signal was observed at the expected size in either the input (lanes 2 or 3) or IP lanes (lanes 5, 6 or 7) but not in the uninduced (lane1) and IgG-Ip lanes (lane 4).
2. **Background:** The presence of a band at unexpected size in either input or IP lanes was not considered towards evaluating the reagent. Such background signal in WB can likely be eliminated using standard techniques but must be evaluated by the end user of these reagents.

Description of individual flags:

1. Significant signal in uninduced input, or poor induction of protein expression:
   Target protein of interest was found to have leaky expression in the uninduced lanes (lane 1) and/or not be visibly increased in expression when comparing the uninduced (lane1) to high induction sample (lane3).

2. Significant signal in IgG or Poor IP:
   Target protein of interest was found to exhibit either an increased propensity to aggregate and/or be unstable. Weak signal might be observed in the IgG lane and/or in the test IP lanes (lane 4 & 5).

3. Low or No signal in FLAG IP:
   Target protein was not recovered using the mouse anti-FLAG (Sigma) IP (lane7). FLAG-tag epitope is likely masked or sterically unavailable to the mAb for IP.

4. Sub-standard IP:
   Under SOP conditions used in this experiment, this reagent was found to perform sub-optimally in its ability to recover the target when compared to input. User will have to empirically determine if alternate protocols can improve recovery of the target protein in IP experiments.

5. Poor WB:
   Under SOP conditions used in this experiment, this reagent was found to perform sub-optimally in its ability to immunoblot the target of interest. User will have to empirically determine if alternate protocols can improve recovery of the target protein in IP experiments.

6. Target protein running substantially heavier or lighter than expected:
   Under SOP conditions used in this experiment, this reagent was found to recognize a target that runs substantially (~20%) heavier or lighter than expected. This occurs even though dose-dependent induction of expression is observed, and sequencing of the expression construct confirms a correctly-sized ORF. It is likely to result from either protein instability or as yet uncharacterized post-translational modifications.