

# Investigating the Hallmarks of Cancer:

## The importance of rigorous reagent validation for research reproducibility

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### ABSTRACT

The Hallmarks of Cancer [1,2] were proposed by Hanahan and Weinberg as a unifying framework to investigate the diverse biological characteristics associated with cancer pathogenesis. Each proposed hallmark represents a phenotypic trait or enabling characteristic considered essential for the malignant transformation of cells. The hallmarks framework was adopted enthusiastically by the scientific community and has enabled a more systematic and unified approach to investigating tumorigenesis. Nevertheless, a significant barrier to research progress remains the pervasiveness of commercial research reagents that fail to meet minimum standards of validation. Cell Signaling Technology, Inc. (CST) has adapted the work by Uhlen, *et al.* [3] to establish practices that ensure rigorous evaluation of antibody functionality, specificity and sensitivity, termed the Hallmarks of Antibody Validation™. Herein, we describe selected antibody reagents targeting proteins that play a role in the manifestation of cancer hallmarks, with a focus on the rigor and biological relevance of the validation methods used in their development.

### CST Hallmarks of Antibody Validation



**Binary Model:** Antibody signal is measured in model systems with known presence/absence of target signal (e.g., wild-type vs. genetic knockout, targeted induction or silencing).



**Ranged Expression:** Antibody signal strength is measured in cell lines or tissues representing a known continuum of target expression levels.



**Orthogonal Data:** Antibody signal is correlated to target expression in model systems measured using antibody-independent assays (e.g., mass spectrometry, *in situ* hybridization).



**Multiple Antibodies:** Antibody signal is compared to the signal observed using antibodies targeting non-overlapping epitopes of the target.



**Recombinant Expression:** Antibody signal is evaluated in cell lines following heterologous expression of native (or mutated) target protein.



**Complementary Assay:** Antibody specificity is validated using complementary assays (e.g., competitive ELISA, peptide dot blots, peptide blocking, or protein arrays).

1. Hanahan and Weinberg 2000: The Hallmarks of Cancer. Cell 100: 55-70.  
2. Hanahan and Weinberg 2011: The Hallmarks of Cancer: The Next Generation. Cell 144: 646-674  
3. Uhlen, *et al.* 2016: A Proposal for Validation of Antibodies. Nature Methods 13: 823-827

