

Tumor Heterogeneity Antibody Selection Checklist

A practical guide for more reliable protein detection in cancer research

Protein detection in cancer research begins with a defined target, but tumor samples often introduce biological complexity that can shape how confidently the result is interpreted. Mixed cell populations, regional variation, tissue processing effects, and changes in target state can all affect antibody performance. Use this checklist to review the variables most likely to influence signal quality, specificity, and confidence in heterogeneous tumor samples.

<p>1. Cellular Composition <i>Who is contributing to your signal?</i></p> <ul style="list-style-type: none"> <input type="checkbox"/> Enrich relevant compartments, such as epithelial versus stromal fractions <input type="checkbox"/> Perform cell sorting, such as FACS or MACS, before protein extraction <input type="checkbox"/> Validate markers in isolated subpopulations <input type="checkbox"/> Include cell-type-specific controls <input type="checkbox"/> Compare bulk versus sorted samples to assess signal dilution 	<p>2. Spatial Sampling <i>Where is your signal coming from?</i></p> <ul style="list-style-type: none"> <input type="checkbox"/> Perform multi-region sampling, including core, margin, and necrotic zones <input type="checkbox"/> Use serial sections for consistency <input type="checkbox"/> Include technical replicates across sampled regions <input type="checkbox"/> Avoid drawing conclusions from a single biopsy alone <input type="checkbox"/> Document sampling location clearly
<p>3. Tissue Processing <i>What is altered during sample preparation?</i></p> <ul style="list-style-type: none"> <input type="checkbox"/> Standardize fixation conditions, including time, reagent, and temperature <input type="checkbox"/> Optimize antigen retrieval protocols <input type="checkbox"/> Check for epitope masking across tissue regions <input type="checkbox"/> Validate staining consistency across sections 	<p>4. Target Validation <i>Can your antibody detect the target under your assay conditions?</i></p> <ul style="list-style-type: none"> <input type="checkbox"/> Verify tissue-specific expression <input type="checkbox"/> Confirm expression in your tumor type <input type="checkbox"/> Map epitope location, such as intracellular versus extracellular <input type="checkbox"/> Confirm membrane topology for transmembrane proteins <input type="checkbox"/> Match the antibody to the intended assay, such as flow cytometry, Western blot, IHC, or IF <input type="checkbox"/> Optimize permeabilization where required
<p>5. Functional State <i>Are you measuring protein presence or protein activity?</i></p> <ul style="list-style-type: none"> <input type="checkbox"/> Pair total protein antibodies with PTM-specific antibodies where relevant <input type="checkbox"/> Include phosphatase and protease inhibitors during sample preparation <input type="checkbox"/> Validate signal under stimulated versus unstimulated conditions <input type="checkbox"/> Perform time-course experiments when appropriate <input type="checkbox"/> Confirm reproducibility of PTM-associated signal 	<p>6. Validation <i>Can you trust the result?</i></p> <ul style="list-style-type: none"> <input type="checkbox"/> Use orthogonal methods, such as RNA, protein, and imaging-based readouts <input type="checkbox"/> Use multiple antibodies targeting different epitopes <input type="checkbox"/> Include positive and negative controls <input type="checkbox"/> Track pre-analytical variables <input type="checkbox"/> Repeat experiments under independent conditions