

**Novel strategies for assaying recombinant antibody function
with high-throughput cell-based assays**

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Phage and related display technologies enable screening of vast repertoires of recombinant human antibodies for isolation of cognate binders to putative disease targets. Screening operations are typically performed on the basis of antibody-target affinity, with subsequent winnowing of candidates on the basis of other antibody-target binding characteristics (such as its ability to prevent native ligand binding). Once the number of antibody candidates is substantially reduced, more descriptive biofunctional data – which ultimately provides the biological basis of antibody potency – are obtained from more descriptive cell-based assays.

As it has been demonstrated that antibody features such as affinity provide a highly unreliable proxy for antibody potency¹⁻³, there is growing need to gain more descriptive potency-correlated (i.e. cell-based) data on individual antibody clones earlier in the antibody discovery process. However, there are a number of factors that confound attempts to obtain this data, particularly in the earliest stages of *E.coli*-based antibody discovery. For example, the direct analysis of *E.coli*-derived recombinant antibody sub-clones is confounded by endotoxins and other contaminants intrinsic to the gram-negative bacteria used in the antibody expression process. In addition, the concentrations of antibody present in *E.coli* preparations are typically of insufficient concentration for use in most cell-based assays. These limitations are most frequently addressed by scaling up the individual growths and using larger-format protein separation and purification tools that do not lend themselves to high-throughput automation – thus creating the present situation where cell-based assays are performed later in the discovery process.

PhyNexus, Inc. PhyTip™ column technology is applied as a high-throughput means for purification and enrichment of recombinant antibodies from small-scale *E.coli* sub-clone preparations that eliminate the need for scale-up, and thus enable true high-throughput cell-based assays of recombinant antibody function. This capability is demonstrated for cell-based assays of antibody antagonist activity via Stat-dependent luciferase reporting of Stat5 phosphorylation. Factors that contribute to PhyTip column and cell-based assay performance are discussed and include column flow and elution conditions, chemical composition of eluents, and optimizing these conditions for the maintenance of cellular viability.

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