

Twist Express Screening Antibodies

Cell-free antibody material for early discovery decisions

Abstract

Twist Express Screening Antibodies have been developed to enable rapid qualitative decision-making and AI model validation. By leveraging expertise in gene synthesis, cell-free protein expression, and binding kinetics assays, Twist Express Screening Antibodies enable the assessment of antibody binding kinetics in as little as 8 business days. Here, we analyze the workflow's metrics, specifically highlighting its ability to produce full-length IgG—a format that has been historically challenging to express using cell-free systems—as well as VHH-Fc and scFv-Fc constructs.

Introduction

Antibody discovery requires rapid experimental feedback to test AI predictions and prioritize candidates. Twist Express Screening Antibodies combine DNA synthesis, cell-free expression, and binding characterization of full-length IgG, VHH-Fc, and scFv-Fc, with capacity for thousands of variants and results in as little as 8 business days from synthesis start, depending on project scope. Here, we assess protein yield, chain-size profiles, binding affinity agreement with cell-based antibodies, and run-to-run reproducibility.

Express Screening Antibody Workflow

The Express Screening Antibody workflow is best suited for rapid evaluation of AI-predicted binding and hit triage. It combines gene synthesis, plate-based cell-free expression, Protein A purification, and SPR binding characterization (**Figure 1**).

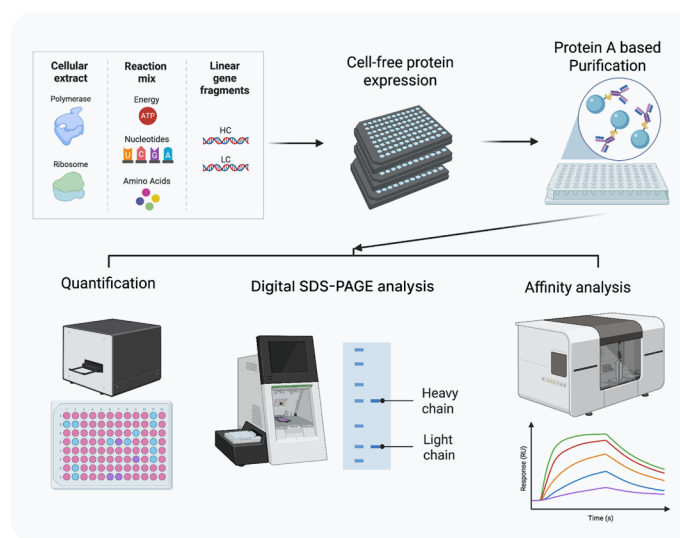


Figure 1. Sequence-to-screen workflow. Expression and affinity capture generate material for concentration measurement, reduced CE-SDS chain-size assessment, and binding characterization.

Results

Screening-grade antibodies bind across three formats

Full-length IgG, VHH-Fc, and scFv-Fc sequences were synthesized on Twist's silicon-based platform and assembled into linear expression templates (LETs) for cell-free protein synthesis.

Cell-free expression generated median yields of 27.6 µg per well for IgG1, 31.1 µg for VHH-Fc, and 16.1 µg for scFv-Fc (**Table 1 & Figure 2**). Across-well coefficients of variation (CVs) were 14%, 22%, and 27%, respectively.

Yield is an A280-derived mass estimate. Because each format

was tested using a different set of antibody designs and experiments, these values describe the tested panels rather than a direct comparison of expression efficiency between formats.

Reduced CE-SDS detected signal at the expected chain sizes in 141 of 146 wells. Of these 141 wells, 67% had more than 80% of detected signal within the expected size windows (**Table 2 & Figure 2**).

Representative antibodies from all three formats showed measurable binding in SPR analyses (**Figure 2**).

Yield from cell-free expression

Format	Yield wells	Median mass / well	Across-well CV
IgG1	48	27.6 µg	14%
VHH-Fc	16	31.1 µg	22%
scFv-Fc	78	16.1 µg	27%

Table 1. Reported yield distributions. (142 wells in total). Mass estimates use A280 concentration and the reported 230 µL eluate basis. These CVs describe variation across a construct panel. The IgG1 panel contains 47 test wells and one control.

Reduced CE-SDS product-window fraction

Format	Product detected	Window fraction, median (IQR)
IgG1	48 / 48 (HC + LC)	84% (80–91%)
VHH-Fc	20 / 20	91% (90–91%)
scFv-Fc	73 / 78	85% (77–97%)*

Table 2. CE-SDS results. (146 wells; 141 with called product signal). *The scFv-Fc fraction is summarized among 73 detected wells. IgG1 and scFv-Fc use blank subtraction.

Screening antibodies produce affinities that agree with cell-based antibodies

SPR-fitted affinities were compared for eight antibody designs produced by cell-free expression and in HEK293 mammalian cells.

Arithmetic-mean KDs were strongly correlated on a log–log scale (Pearson $r = 0.973$) (**Figure 3**). Seven of eight cell-free KDs were within threefold of the HEK293 values. IgG-5's mean KD was approximately tenfold lower in cell-free material. It should be noted that the available results cannot distinguish assay effects from a source-related difference.

These paired results support comparable binding affinities for the antibodies tested. Lower recovered yield or a noisy CE-SDS profile does not, by itself, determine binding performance.

Screening grade antibodies produce consistent kinetic data

To test reproducibility, two de novo design panels (57 IgG1 and 86 VHH-Fc designs) plus 15 reference binders were expressed and screened by SPR in two independent runs. Binding calls agreed design-for-design except for two antibodies at the edge of the measurable range, and 19 of the 21 antibodies that resolved in both runs gave KDs within threefold across 0.4 nM to 2.9 µM (**Figure 4**).

Use case: hit prioritization

In one researcher-initiated project, 57 designs—47 IgG1 and 10 VHH-Fc—were screened against a common target. SPR returned 1:1 kinetic fits for 53 of 57 designs: 43/47 IgG1 and 10/10 VHH-Fc (**Figure 5**). IgG1 KDs spanned 0.5 nM to 2.3 µM (median 18 nM) and VHH-Fc KDs 16 to 224 nM (median 29 nM). Because the KDs form a continuous rank order, the data support both top-n selection and threshold-based deselection.

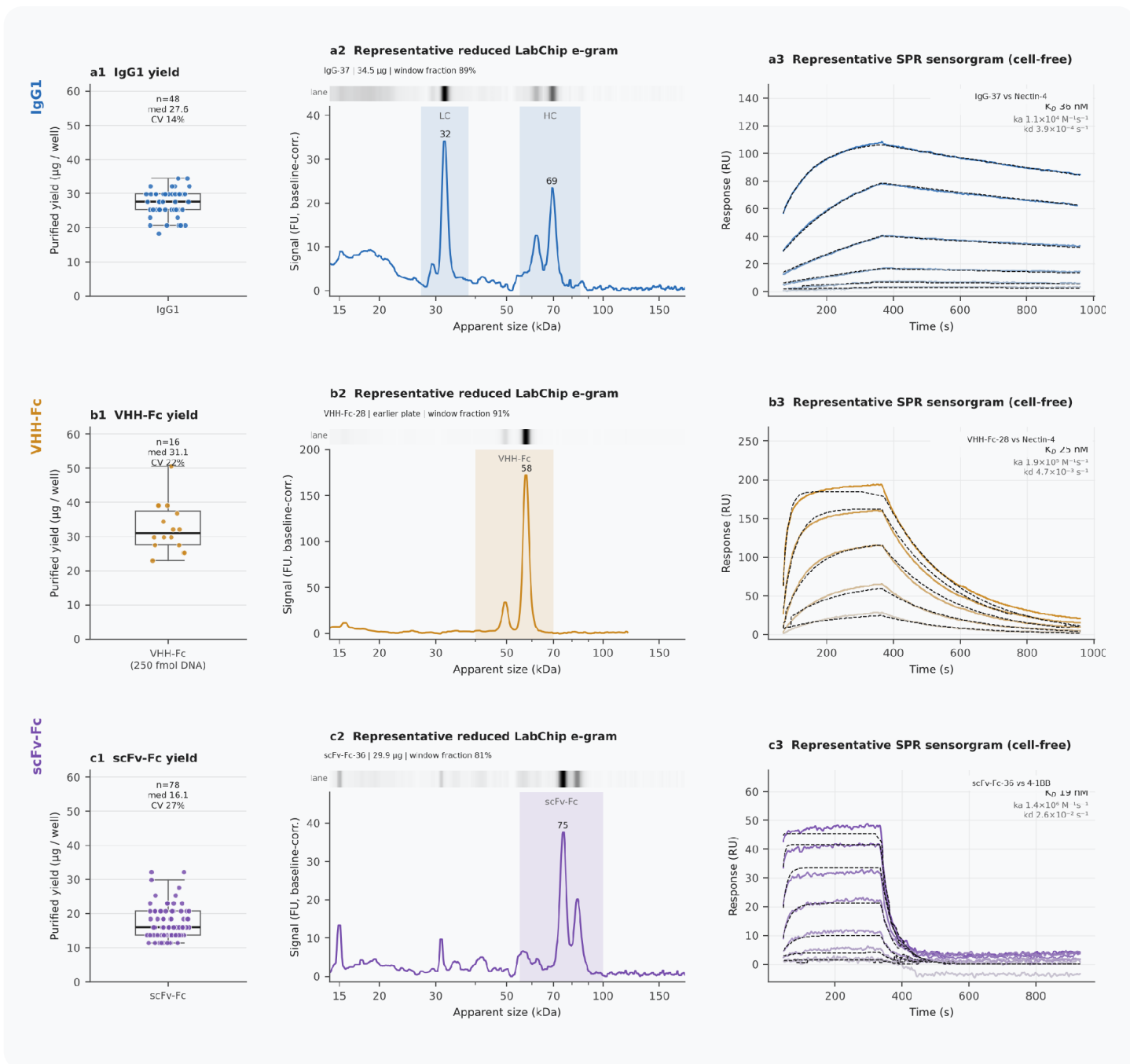


Figure 2. All three formats express, run at the expected size and bind their target. In each row, the CE-SDS profile and the sensorgram come from the same antibody. Rows show IgG1 (a), VHH-Fc (b) and scFv-Fc (c). Left: A280-derived recovered mass, individual wells, median and IQR. Middle: representative baseline-corrected reduced CE-SDS profiles; shading denotes desired product size windows, with peaks corresponding to pixel density in the virtual gel lane (shown above each profile). Right: Carterra analysis-exported sensorgrams, digitized from the instrument rendering rather than exported numerical traces. Colored curves reproduce measured responses and dashed curves the reported 1:1 fit. The reported KDs are 36 nM (IgG-37), 25 nM (VHH-Fc-28) and 19 nM (scFv-Fc-36).

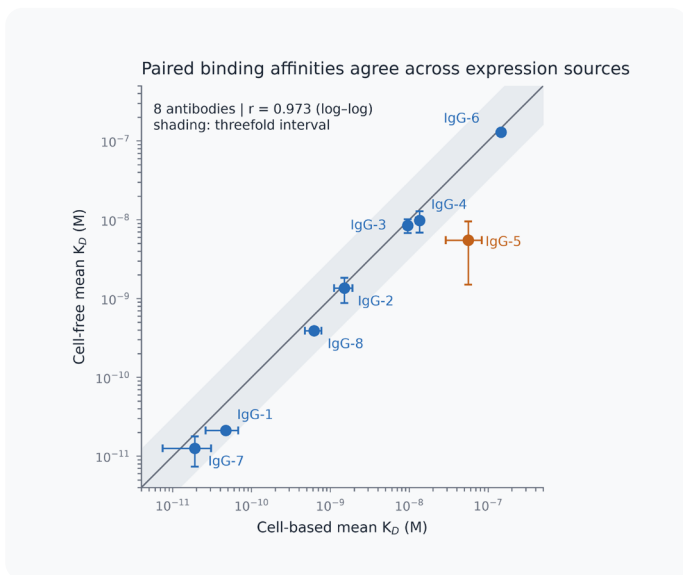


Figure 3. Cell-free antibodies reproduce HEK293-derived affinities.

Arithmetic-mean K_D by source for the eight antibodies with paired SPR estimates, with ± 1 SD of the fitted estimates; the diagonal denotes equality and shading the threefold interval. Three fitted estimates per antibody and source except IgG-7 HEK293 ($n = 2$); these are within-assay estimates, not independent production-batch replicates, and immobilization levels differ between sources. IgG-5 (orange) falls outside the interval.

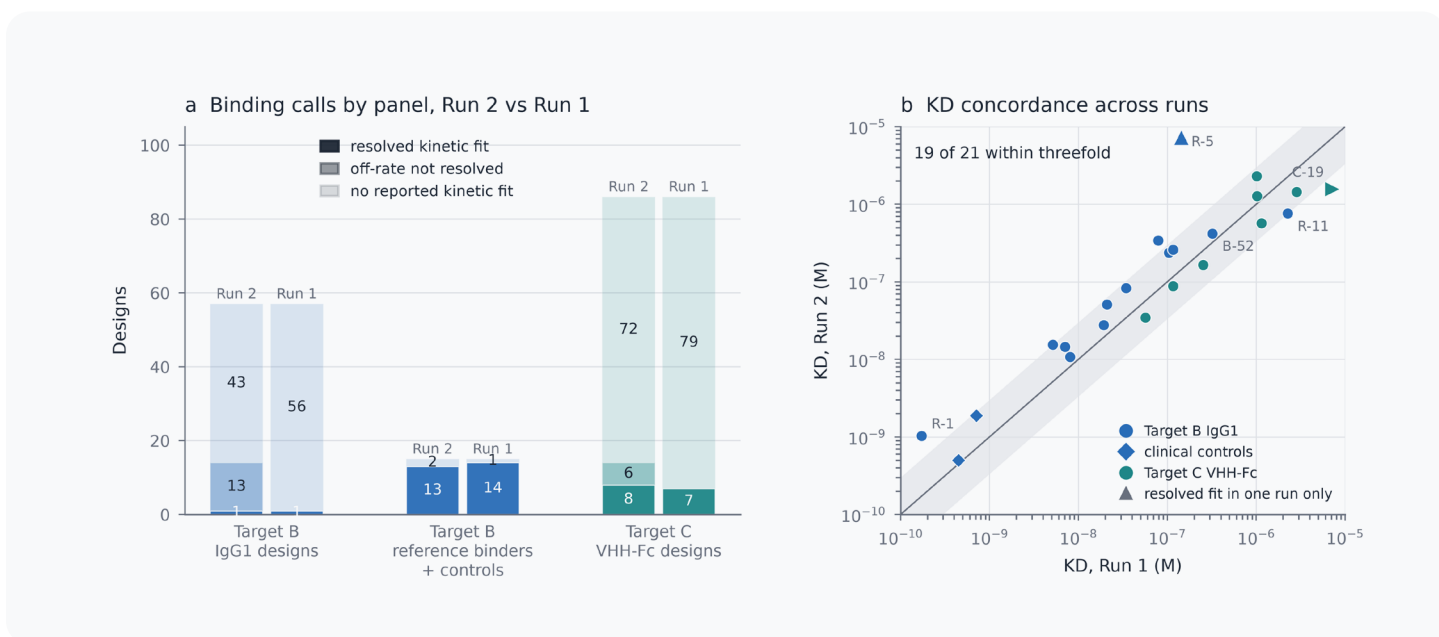


Figure 4. Independent repeat screens give concordant binding calls and affinities.

(a) Binding calls in Run 2 (left bar of each pair) and Run 1 (right bar) for IgG designs (Target B designs) as well as 13 reference binders and two clinical controls. VHH-Fc designs (Target C designs) are shown in green. (b) Fitted K_D in Run 2 (vertical axis) versus Run 1 (horizontal axis) for every antibody that gave a resolved fit in both runs; the diagonal is equality and the band the threefold interval. Triangles on the axes mark the two antibodies with resolved fits in one run only. "Off-rate not resolved" denotes a reported fit whose dissociation rate could not be determined reliably; these fits are excluded from the affinity comparison.



Figure 5. SPR returns 1:1 kinetic fits for 53 of 57 designs (43/47 IgG1 and 10/10 VHH-Fc). (a) IgG1 and (b) VHH-Fc designs, one panel per design, ordered by variant number. Solid blue or teal traces show digitized measured responses; dashed orange curves show the reported 1:1 fits. The six-point analyte series was 4.1, 12.3, 37.0, 111, 333 and 1,000 nM (threefold dilutions). Fitted KDs are given at the top right of each panel; “n/a” denotes no reported kinetic fit. Sensorgrams were redrawn from instrument fitted-report graphics.

Conclusion

The Twist Screening Grade workflow supports rapid antibody screening across full-length IgG, VHH-Fc, and scFv-Fc, with results in as little as 5 business days, depending on project scope. Binding affinities agreed with cell-based antibodies in the paired study, and independent screens showed consistent binding calls and affinities. Together, these results support high-throughput binding data generation for AI training and fast hit selection, with selected candidates advancing to further characterization.

Questions?

Get in touch at sales@twistbioscience.com or learn more at twistbioscience.com

For Research Use Only. Not for use in diagnostic procedures.

DOC-4096 REV 1