Appendix 7. Antibody Track Experimental Testing Procedure and Scoring Criteria

The Antibody Track focuses on antibody design and screening for the target PVRIG. The task objective is to design antibody molecules that bind PVRIG and have the potential to block its interaction with the natural ligand PVRL2. The competition uses a multi-round, stepwise evaluation mechanism. It systematically evaluates sequence design, experimental producibility, functional activity, and oral presentation and defense performance to select outstanding Participating Teams and candidate molecules with binding activity, blocking activity, and model iteration capability.

The overall process includes five stages: Registration, Preliminary Round, Iterative Submission, Semifinal Round, and Final Round. Both the Preliminary Round and the Semifinal Round include two full wet-lab evaluation steps: Initial Screening and Secondary Screening. The results serve as the basis for advancing teams to the next stage. Before the Semifinal Round, advancing teams must resubmit 50 candidate antibody sequences during Iterative Submission. These 50 sequences may include sequences submitted in the Preliminary Round or newly optimized or redesigned sequences based on Preliminary Round results.

I. Common Scoring Workflow for the Preliminary Round and Semifinal Round

The Preliminary Round and the Semifinal Round will follow the same technical evaluation path:

1. Initial Screening: Evaluation based on antibody expression yield, purity, and single-concentration BLI binding results.

2. Secondary Screening: Evaluation based on BLI multi-concentration affinity measurement and IC_{50} values obtained from competitive ELISA assays.

The evaluation results at each stage will be used as the basis for candidate antibody molecules entering subsequent experimental steps and for Participating Teams advancing to subsequent stages. After the Preliminary Round, the Organizer will determine the list of teams eligible for Iterative Submission based on the comprehensive results of the Secondary Screening step. Each team will be represented by its best-performing candidate antibody molecule. After Iterative Submission and Semifinal shortlisting review, the relevant Participating Teams will enter the Semifinal Round. After the Semifinal Round, the Organizer will determine each team's wet-lab score based on Secondary Screening results and will use these results to determine the teams advancing to the Final Round oral presentation and defense.

II. Initial Screening Step

The Initial Screening step is designed to conduct standardized recombinant expression and purification of candidate antibody sequences shortlisted during the Registration Stage. The candidate antibody molecules obtained by expression will then undergo preliminary in vitro binding validation. This step evaluates expression feasibility, sample quality, and preliminary PVRIG binding, and selects candidates for Secondary Screening. Compared with the shortlisting review at the Registration Stage, which is mainly based on sequence-level evaluation, Initial Screening introduces experimental data and focuses on the practical performance of candidate molecules in preparation and initial functional testing.

1. Overall Process

Candidate antibody sequences that pass the shortlisting review during the Registration Stage will enter the recombinant expression and experimental Initial Screening workflow. The Organizer will

standardize all shortlisted sequences, including codon optimization, unified expression vector construction, and unified testing procedures. This is intended to reduce systematic differences and improve comparability across candidate molecules. After expression, purification, and single-concentration BLI testing, the Organizer will conduct comprehensive scoring and ranking based on expression yield, purity, and binding results. Candidate antibody molecules will then be selected for the Secondary Screening step.

2. Expression and Construct Requirements

All shortlisted antibody sequences will be constructed for recombinant expression by the Organizer. Conventional antibodies will generally be constructed on a human IgG1 backbone. If the candidate antibody is in single-domain antibody (VHH) format, it will be constructed as a VHH-hFc fusion protein. To improve expression efficiency and consistency, all candidate antibody sequences will undergo codon optimization before expression. Codon optimization, vector construction, and expression system selection will be performed by the Organizer under a unified procedure. Once a submitted sequence is confirmed, individualized expression condition adjustments will generally not be accepted.

Expression will be performed in a 4 mL or 10 mL CHO transient expression system. The Organizer will select the applicable expression volume based on experimental scheduling and sample conditions. After expression, one-step affinity purification, such as Protein A affinity purification, will be used to obtain antibody samples for subsequent testing.

3. Expression Yield and Purity Evaluation

The Initial Screening step will evaluate the expression performance of candidate antibody molecules. Expression results will be based on the actual amount of purified antibody obtained and converted to yield (mg/L) according to the culture volume. In principle, a candidate antibody molecule with a yield of at least 25 mg/L will be considered to meet the basic testing requirement. For a 4 mL CHO expression system, at least 100 μg of purified antibody should generally be obtained to support subsequent purity analysis and single-concentration BLI testing. For candidate antibody molecules with clearly insufficient expression yield or insufficient sample amount to support complete testing, the Organizer may lower their comprehensive ranking or, if necessary, exclude them from the next evaluation step.

After purification, the Organizer will conduct quality testing of candidate antibodies, with a focus on sample purity and major impurity profiles. Testing methods include but are not limited to SDS-PAGE and HPLC. In principle, SDS-PAGE purity and HPLC purity of candidate antibody molecules are both recommended to be at least 80%. These indicators are used to evaluate quality risks such as aggregation, degradation, or residual impurities that may affect subsequent analysis. For candidate antibody molecules with lower purity that can still be tested, the Organizer will make a comprehensive judgment based on expression yield and binding results. For molecules with clearly substandard purity, poor sample quality, or insufficient testing reliability, the Organizer may lower their ranking or, if necessary, exclude them from advancement.

4. Single-Concentration BLI Initial Screening

After expression and purification are completed and the samples are confirmed to meet basic testing requirements, the Organizer will use single-concentration Bio-Layer Interferometry (BLI) to preliminarily screen the binding ability of candidate antibody molecules to PVRIG. This step is intended to rapidly identify candidate antibody molecules with clear binding signals and to distinguish non-binding, weak-binding, and binding molecules. The results will support subsequent BLI multi-concentration affinity measurement and competitive ELISA testing.

This step will use a Protein A biosensor capture format. Candidate antibody molecules will be immobilized on the sensor surface, and their association and dissociation behavior with soluble human PVRIG antigen will be measured.

Single-concentration BLI results will generally be classified into three categories: binding, weak binding, and no binding. At an antigen concentration of no more than 500 nM, the BLI response shift (nm) will be used as the decision indicator. A response shift ≥ 0.1 nm is defined as binding; 0 ≤ response shift < 0.1 nm is defined as weak binding; and response shift < 0 nm is defined as no binding. The final result will be determined by considering response signal strength, curve shape, fitting quality, and sample quality.

5. Composite Ranking and Advancement Principles

The Initial Screening step will use quantitative scoring and weighted ranking based on single-concentration BLI binding results, expression yield, and purity. The scoring rules are as follows:

1. BLI binding score: binding = 100 points; weak binding = 50 points; no binding = 0 points.

2. Expression yield score: >100 mg/L = 100 points; 25-100 mg/L = 60 points; <25 mg/L = 0 points.

3. Purity score: purity <80% = 60 points; purity 80%-90% = 80 points; purity ≥90% = 100 points.

4. Composite score = BLI score × 0.7 + expression yield score × 0.2 + purity score × 0.1.

The Organizer will rank candidate antibody molecules based on their composite scores. The number of candidate antibody molecules advancing to Secondary Screening in the Preliminary Round and the Semifinal Round will follow the corresponding schedule rules. In the Preliminary Round, the top 200 candidate antibody molecules by composite score will generally be selected for Secondary Screening. In the Semifinal Round, the top 15 teams will generally be selected, and from these teams, no more than 10 candidate antibody molecules per team and no more than 150 candidate antibody molecules in total will enter Secondary Screening.

6. Additional Notes

1. The single-concentration BLI results obtained in Initial Screening are used only for rapid screening. They are not equivalent to final precise affinity conclusions. The final binding ability and blocking potential of candidate antibody molecules must be further confirmed through multi-concentration experiments in Secondary Screening.

2. For molecules that cannot produce complete testing results due to insufficient sample amount, substandard purity, or abnormal experimental signals, the Organizer will decide based on actual circumstances whether to include them in the ranking or treat them as ineligible for the next step.

III. Secondary Screening Step

The Secondary Screening step is designed to conduct higher-precision binding and functional validation of candidate antibody molecules that pass Initial Screening. It will focus on true binding affinity to PVRIG and the ability to block the PVRIG-PVRL2 interaction. The results will be used to identify the best-performing candidate molecules and to support team advancement to the next stage. Compared with Initial Screening, Secondary Screening uses BLI multi-concentration affinity measurement and competitive ELISA assays to compare and rank candidate molecule activity more systematically and stringently.

Candidate antibody molecules that pass Initial Screening will enter a unified Secondary Screening workflow. The Organizer will use a unified experimental platform, reagent conditions, and data analysis standards to conduct BLI multi-concentration affinity measurement and competitive ELISA

assays for all candidate antibody molecules. The results will serve as the core basis for evaluating binding activity, blocking ability, and overall competitiveness. They will also be used for team advancement, wet-lab score calculation, and subsequent award determination.

1. BLI Multi-Concentration Affinity Measurement

The Secondary Screening step will use multi-concentration BLI to measure the binding affinity between candidate antibody molecules and PVRIG. The principle and basic workflow are the same as the single-concentration BLI method used in Initial Screening. Candidate antibodies will be captured using Protein A biosensors, and their association and dissociation behavior with soluble human PVRIG antigen will be measured. Unlike Initial Screening, Secondary Screening will use five antigen concentration gradients to obtain more stable and accurate kinetic data. Kd values will be calculated based on full-concentration fitting results.

Under unified experimental conditions, the Organizer will fit and analyze the multi-concentration binding curves of all candidate molecules and rank them based on fitted Kd values. In principle, a lower Kd value indicates stronger affinity and a higher ranking. Kd ranking will be in ascending order. The candidate molecule ranked 1st will receive 100 points. Each subsequent rank will receive 2 points less than the previous rank. This score will be used in the track composite scoring. If the Kd of a candidate molecule cannot be fitted from multi-concentration data, or if the molecule is determined to be non-binding, the score for this item will be 0 points.

2. Competitive ELISA Assay

Secondary Screening will further use an in vitro blocking assay to test the ability of candidate antibodies to block the binding of human PVRIG to its natural ligand human PVRL2. The corresponding IC_{50} value will be calculated. This assay directly evaluates the blocking effect of candidate antibodies on the functional site of the target and is an important basis for assessing functional activity.

The competitive ELISA assay may be conducted as follows. Human PVRIG recombinant protein is coated onto a 96-well microplate. After the candidate antibody fully binds to and occupies the relevant epitope, His-tagged human PVRL2 protein is added. The His-tag signal is then measured to calculate the amount of PVRIG-PVRL2 binding and to analyze the blocking effect of the candidate antibody. The IC_{50} value is then calculated. In practice, the Organizer may reasonably adjust specific experimental parameters and operating details based on experimental conditions, sample status, and pre-experiment results. Such adjustments will not change the basic principle or evaluation objective of this test.

The Organizer will rank candidate antibody molecules based on their IC_{50} values. In principle, a lower IC_{50} value indicates stronger blocking activity and a higher ranking. IC_{50} ranking will be in ascending order. The candidate molecule ranked 1st will receive 100 points. Each subsequent rank will receive 2 points less than the previous rank. If a candidate molecule shows no competitive activity, the score for this item will be 0 points.

3. Composite Scoring

The Secondary Screening composite score will focus on binding affinity and functional blocking activity. The composite score consists of the Kd ranking score and the IC_{50} ranking score, with weights of 0.5 and 0.5, respectively.

Composite score = Kd ranking score × 0.5 + IC_{50} ranking score × 0.5.