MPSA-AB5000: A high-throughput cell-based membrane protein screening array for accurate early-stage off-target assessment

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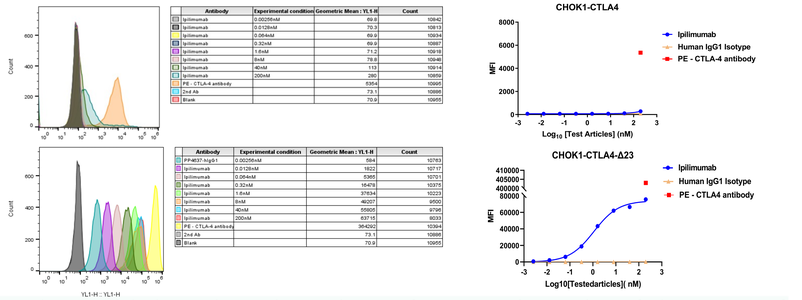
Abstract

Antibody drug development is lengthy and complex, often hindered by various challenges in clinical advancement. Reports indicate that off-target binding of antibodies may be a significant issue, with approximately 30% of preclinical candidates potentially experiencing these off-target problems. With the rise of new modalities such as CD3 bispecific antibodies, ADCs, and CAR-T therapies, preliminary off-target screening of antibodies has become increasingly important.

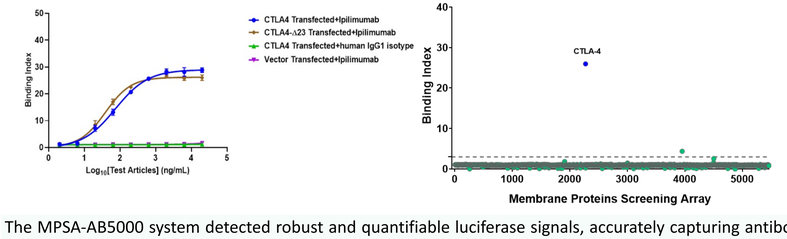
To achieve high sensitivity, MPSA-AB5000 uses a luciferase reporter cell line. This is especially effective for targets like CTLA-4, which rapidly internalize and show low surface expression. Thanks to signal amplification in the reporter system, even minimal antibody–target binding can be converted into a clear, quantifiable luminescent signal. The MPSA-AB5000 covers diverse human membrane proteins, expressed in live cells to preserve native conformations. Also, we have established an industry-leading HEK-293T knockout (KO) cell library covering key targets (e.g., B7H3, ADAM9, EGFR), which effectively eliminates endogenous interference during off-target screening.

MPSA-AB5000 supports both antibody and CAR-T off-target screening. For CAR-T, the CAR is directly expressed on the surface of reporter cells, enabling cell-based specificity assessment that closely mimics its in vivo mechanism. Compared with traditional CAR fusion protein approaches, this method more accurately reflects specificity and better represents physiological conditions.

Specificity screening of CTLA-4 antibody

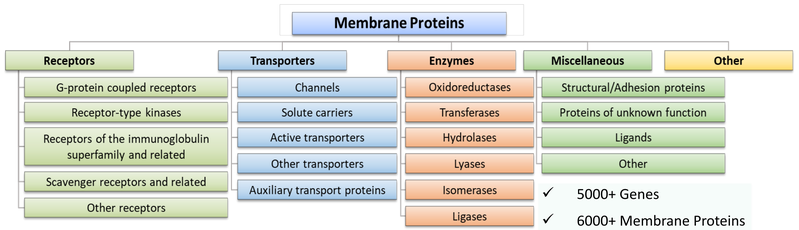


FACS detection fails due to the rapid internalization of CTLA-4, resulting in the swift disappearance of membrane-bound antigens and the inability to effectively capture antibody binding signals.



The MPSA-AB5000 system detected robust and quantifiable luciferase signals, accurately capturing antibody–antigen interactions despite rapid internalization.

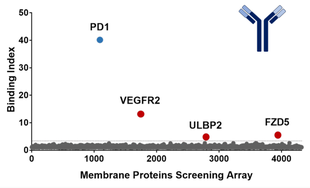
6000+ Full Coverage Membrane Protein



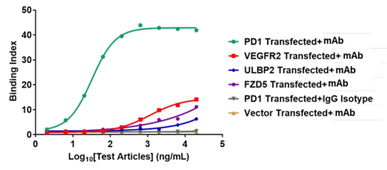
✓ 5000+ Genes
✓ 6000+ Membrane Proteins

Case studies: specificity screening of monoclonal antibodies

Target Screening: PD1



Co-culture Dose-response Confirmation

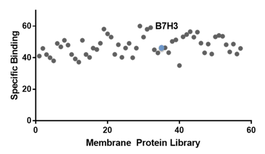


✓ VEGFR2 and ULBP2 identified as off-targets for SHR1210, an anti-PD1 mAb, in AB5000 platform. (Approved, mAb)

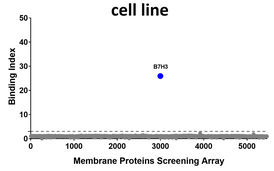
✓ Consistent with the literature that anti-PD1 "SHR-1210" off-targets VEGFR2, ULBP2 cause side effect such as capillary hemangioma (Finlay et al., MAbs, 2019)

293T knockout (KO) cell library covering 80+ targets

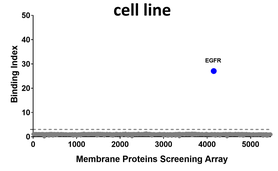
B7H3 mAb tested in 293T cells



B7H3 mAb tested in 293T-B7H3-KO cell line



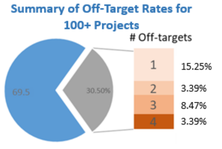
EGFR mAb tested in 293T-EGFR-KO cell line



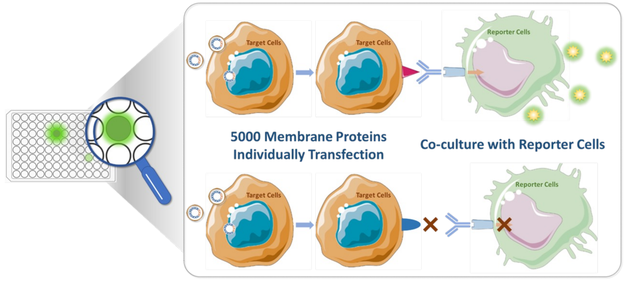
High B7H3 expression in HEK293T cells contributes to significant background interference. Adopting B7H3-KO effectively reduces background and improves assay sensitivity.

Off-target mAb binding is common and a likely cause of drug failure

1 in 3 to 4 mAbs is polyspecific across 100+ projects



Off-target risks differ among antibodies: some bind proteins within the same family, while others unexpectedly interact with structurally unrelated molecules, highlighting the diverse nature of unintended binding.

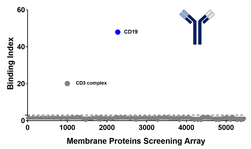


✓Full coverage of human membrane proteins
✓High sensitivity
✓Natively expressed in live cells
✓Ultrashort turnaround

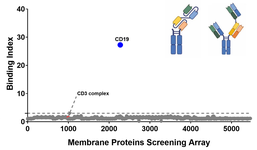
Testing TCE molecule antibodies: CD19 x CD3

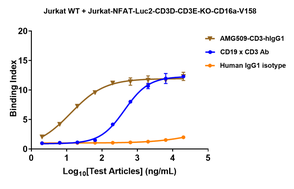
CD19 serves as a specific identification marker for B cells and their malignant clones (tumor cells). CD19 × CD3 bispecific antibodies have achieved breakthroughs in the field of hematological malignancies and have now successfully expanded into the treatment of autoimmune diseases, becoming one of the core tools for clearing pathogenic B cells.

Case I



Case II

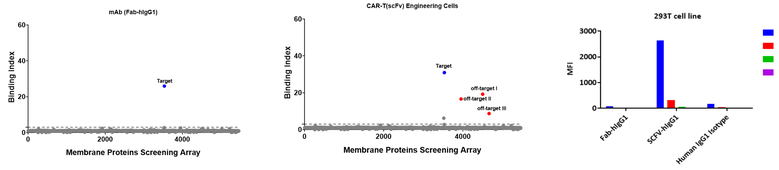




✓ Jurkat-NFAT-Luc2-CD3D-CD3E-KO-CD16a-V158: Reporter cell line for TCE antibody testing. This multifunctional cell line combines NFAT-Luc2 signaling detection, CD3 receptor knockout, and CD16a V158 FcγR functional verification, making it an ideal model for screening and optimizing bispecific antibodies, immunomodulatory antibodies, and Fc-engineered antibodies.

✓ The distinction in CD3-complex recognition between conventional TCE (Constitutive TCE) and conditional activation TCE (Weak affinity for CD3) during off-target screening.

Off-Targets of CAR-T reporter cell



The scFv form of antibodies increases the possibility of off-targets binding.

One of the primary drawbacks of scFv antibodies is their increased likelihood of non-specific binding. This occurs because the flexible linker can cause the VH and VL domains to adopt fewer stable conformations, leading to interactions with unintended targets.

Why We Choose the MPSA-AB5000

Natively expressed in live cells
- Ensure native protein conformation
- Three cell line options can be used to minimize background

High sensitivity reporter cell detection
- Wash-free strategy reduces false negatives
- Signal amplification makes large discrimination
- High throughput microplate system guarantees best stability and creditability

Successfully screened off-target molecules
- Ultrashort turnaround for data report
- Competitively priced