

Protocol

INTRODUCTION

Some antibodies can recognize more than one antigen, which is referred to as polyspecificity (Van Regenmortel, M.H.V., 2014). Therapeutic antibodies have different half-lives in the body that can affect their therapeutic efficiency. One reason for fast clearance from the body is the polyspecificity of the antibody. An ELISA assay using non-specific binding to baculovirus particles (BVPs) of therapeutic antibodies showed correlation with the fast *in vivo* clearance of antibodies (Hötzel et al., 2012). Determining the polyspecificity of an antibody early on during therapeutic antibody development helps with decision making whether to pursue the antibody therapy further.

DESCRIPTION

Baculovirus particles (BVPs) can be used to measure polyspecificity of therapeutic antibody candidates in *in vitro* assays. This assesses the viability of an antibody as a therapy early on. Pre-coated BVP ELISA plates are ready to use 96-well strip-well plates coated with BVP and immobilized on the well surface. The plates are supplied in a dry format for easy storage and shipment.

COMPONENTS INCLUDED

One clear high-binding, flat-bottom polystyrene microplates (360 µL/well) with pre-coated BVP on the well surface. Each microplate consists of twelve removable strips of 8 wells and a frame.

REFERENCES

Van Regenmortel, M.H., "Specificity, polyspecificity, and heterospecificity of antibody-antigen recognition", *J Mol Recognit*, 2014. 27(11): p. 627-39.

Hötzel *et al.*, "A strategy for risk mitigation of antibodies with fast clearance", *mAbs*, 2012, 4:6, 753-760.

Xu *et al.*, "Addressing polyspecificity of antibodies selected from an *in vitro* yeast presentation system: a FACS-based, high-throughput selection and analytical tool.", 2013, *Protein Engineering, Design & Selection*, 26:10, pp. 663-670.

PROTOCOL

1. Add 100 µL of blocking buffer (PBS with 0.5% BSA) and incubate for 1 hr.
2. Wash 3 times with 100 µL of PBS.
3. Add 50 µL of 3-6 µg/mL primary antibody and incubate for 1 hr.
4. Wash 6 times with 100 µL of PBS.
5. Add 50 µL of anti-Human-IgG-HRP and incubate for 1 hr.
6. Wash 6 times with 100 µL of PBS.
7. Add 50 µL of TMB substrate and incubate for 10-15 min.
8. Stop the reaction with 50 µL of 2M sulfuric acid.
9. Read absorbance at 450 nm.
10. BVP score is determined by normalizing absorbance by control wells with no test antibody.