

PRODUCT DATASHEET

AVA00106

Anti-Trastuzumab Affimer[®] Reagent – 6xHis tag, Cysteine

Target: Trastuzumab
Avacta clone ID: 386_737_B3

Version: 02
Last revised: May 2018

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC APPLICATIONS.

Applications: ELISA

Cross-reactivity: None to Avastin,
Humira, Rituximab, or human IgG

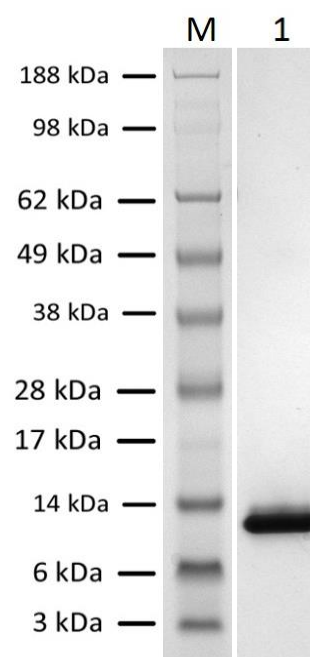
Storage: 4°C

Product Background:

Highly specific Affimer[®] reagent against the humanised drug Trastuzumab (Herceptin). Comparable performance described compared to its cognate ligand, Her2 by ELISA. Type I (Anti-idiotypic) Affimer[®] reagent for use in ELISA.

Product Information:

Form	Liquid
Storage Instructions	Store at 4°C for up to 6 months. For longer term storage, it is recommended to store at -20°C in single use aliquots.
Buffer	100 mM Sodium Citrate, 150 mM Sodium Chloride, 5 mM TCEP, 0.02% Sodium Azide, pH 6.5
Purity	>95%
Purification method	IMAC followed by Size Exclusion Chromatography
Clonality	Monoclonal
Format	C-terminal 6xHis tag and Cysteine



Source/Purification:

This monoclonal Anti-Trastuzumab Affimer[®] Reagent was produced by IPTG-induced expression in *E. coli* and purified from cell lysates using IMAC chromatography followed by size exclusion chromatography. Purity is >95% (see SDS-PAGE image, right). (M = protein marker, 1 = Anti-Trastuzumab Affimer[®] Reagent)

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Specificity and Sensitivity:

Assay Parameters	
Sensitivity	30 ng/mL
Dynamic range	30-1600 ng/mL
Intra-assay precision (CV)	<10%
Bias	≤9.6%
Inter-assay precision (CV)	8.6%
Bias	≤4.5%

Anti-Trastuzumab Affimer® Reagent shows excellent sensitivity to the therapeutic antibody target comparable to the natural ligand, HER2. Capture ELISA data using anti-trastuzumab Affimer® reagents passively adsorbed onto ELISA plate showed detection of the trastuzumab antibody target over the clinically relevant concentration range 30-1600 ng/mL, performing in line with the natural HER2 ligand, to a lower concentration of 30 ng/mL.

Application Example - ELISA:

Affimer® reagent coating: Maxisorp plates coated with 100 µL/well Anti-Trastuzumab Affimer® Reagent (1 µg/mL) in 1 x Carbonate/Bicarbonate buffer, pH 9.6 (16 h, 4°C)

Blocking: Blocked 300 µL/well in 1 x Casein Block (Sigma) in 1 x PBS (2 h, room temperature (RT))

Wash 1: 3 x 300 µL/well of 1 x PBS-T (0.05% Tween-20) using plate washer

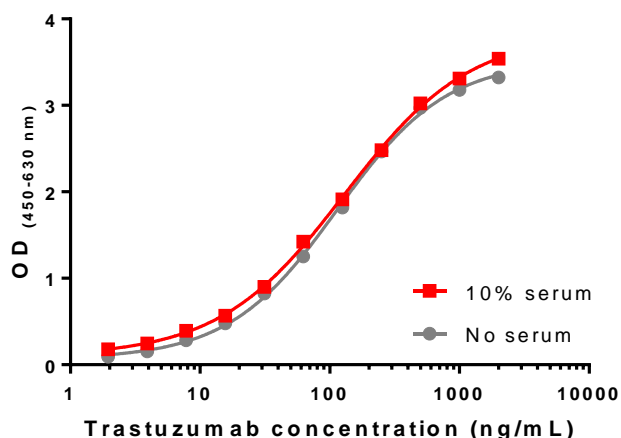
Sample incubation: Dilute sample 1/10 (MRD), 1 x Casein Block (Sigma) in 1 x PBS (1 h, RT)

Wash 2: 3 x 300 µL/well of 1 x PBS-T (0.05% Tween-20) using plate washer

Detection: 1 in 100,000 anti-human IgG Fc HRP (Bethyl A80-319P) in 1 x Casein Block (Sigma) in 1 x TBS (1 h, RT)

Wash 3: 3 x 300 µL/well of 1 x PBS-T (0.05% Tween-20) using plate washer

Substrate: TMB (Surmodics)



Capture ELISAs demonstrate the anti-trastuzumab Affimer® reagent specifically identifies the therapeutic antibody at clinically relevant concentrations against a complex human serum background. No significant difference was observed in target sensitivity between the buffer and serum matrices.