

PRODUCT DATASHEET

AVA00109

Anti-Ipilimumab Affimer[®] Reagent – HA, 6xHis tag

Target: Ipilimumab
Avacta clone ID: 433_638869

Version: 01
Last revised: May 2018

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

Applications: ELISA

Cross reactivity: None to human IgG,
Humira or Rituximab

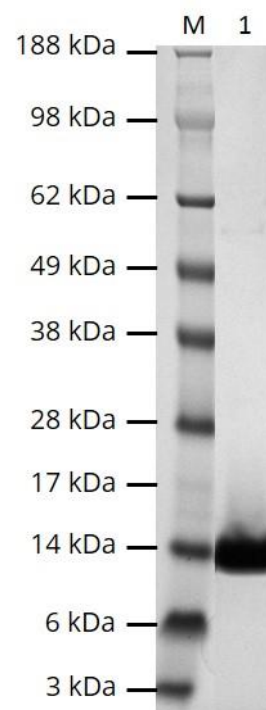
Storage: 4°C

Product Background:

Highly specific Affimer[®] reagent against the humanised drug Ipilimumab. Comparable performance to its cognate ligand, CTLA-4 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 Phosphate, 150 mM Sodium Chloride, 0.02% Sodium Azide, pH 7.4
Purity	>95%
Purification method	IMAC followed by Size Exclusion Chromatography
Clonality	Monoclonal
Format	C-terminal HA and 6xHis tag



Source/Purification:

This monoclonal Anti-Ipilimumab 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-Ipilimumab Affimer[®] reagent).

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Specificity and Sensitivity:

Assay Parameters	
Sensitivity	15 ng/mL
Dynamic range	15-1000 ng/mL
Intra-assay precision (CV)	0.3%-12%
Intra-assay recovery	91.7-122.6%*
Inter-assay precision (CV)	2.7-11.4%
Inter-assay recovery	94.4-110.9%

Anti-Ipilimumab Affimer[®] reagent shows excellent sensitivity to the therapeutic antibody target where performance is comparable to its target, CTLA-4. Capture ELISA data using Anti-Ipilimumab Affimer[®] reagent passively adsorbed onto ELISA plates showed detection of Ipilimumab antibody over the clinically relevant concentration range 15-1000 ng/mL.

* Values approaching FDA limits were obtained at a LOQ.

Application Example - ELISA:

Affimer[®] reagent coating: Maxisorp plates coated with 100 μ L/well Anti-Ipilimumab Affimer[®] reagent (1 μ g/mL) in 1 x Carbonate/Bicarbonate buffer, pH 9.6 (16 h, 4°C)

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

Wash 1: 3 x 300 μ L/well of 1 x PBS-T (0.05% Tween-20)

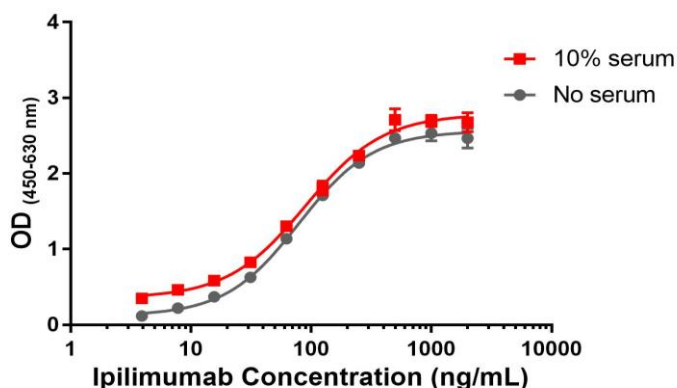
Sample incubation: Prepare standard curve in 1 x Casein Block (Sigma) in 1 x PBS (1 h, RT)

Wash 2: 3 x 300 μ L/well of 1 x PBS-T (0.05% Tween-20)

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

Wash 3: 3 x 300 μ L/well of 1 x PBS-T (0.05% Tween-20)

Substrate: TMB (Surmodics)



Capture ELISAs demonstrate the Anti-Ipilimumab 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.