

# BioSim™ anti-Cetuximab (Erbix®) (Human) ELISA Kit

rev 04/20

(Catalog # E4391-100, 100 assays, Store at 4°C)

## I. Introduction:

Cetuximab (Erbix®) is a chimeric monoclonal antibody of the immunoglobulin G1 (IgG1) and FDA-approved epidermal growth factor receptor (EGFR) inhibitor. Preclinical studies have shown that cetuximab enhances the antitumor effects of chemotherapy as well as radiotherapy by inhibiting cell proliferation, angiogenesis and metastasis and by promoting apoptosis is used for the treatment of metastatic colorectal cancer, metastatic non-small cell lung cancer and head and neck cancer. Cetuximab blocks growth factor-induced activation of the downstream mitogen-activated protein kinase, inhibiting cell proliferation. It also decreases angiogenic factors, inhibits tumor-cell invasion and metastasis via downregulation of matrix metalloproteinases (MMPs) and VEGF, and promotes apoptosis by upregulating apoptotic protein, Bax, with the help of other chemotherapeutic agents. However, some patients develop unwanted immunogenicity, which leads to production of anti-drug-antibodies (ADAs) inactivating the therapeutic effects of the treatment and, in rare cases, inducing adverse effects. BioVision's BioSim™ anti-Cetuximab ELISA kit is designed to quantify/measure the antibody against Cetuximab with high specificity and sensitivity in biological matrices.

## II. Application:

This ELISA kit is used for *in vitro* quantitative determination of antibody against Cetuximab in serum and plasma

Detection Range: 62.5 - 500 ng/ml

Sensitivity: 62.5 ng/ml

Assay Precision: Intra-Assay: CV < 30%; Inter-Assay: CV < 30% (CV (%) = SD/mean X 100)

Cross Reactivity: Cetuximab (Erbix®) infusion camouflages/masks the presence of antibody to cetuximab (ATC) in serum/plasma samples. Therefore, blood sampling time is critical for detection of ATC. It is convenient to obtain blood sample just before the infusion or at least 2 weeks after the infusion of Cetuximab.

Recovery rate: <100±30% with normal human serum samples with known concentrations

## III. Sample Type:

Human serum and plasma

## IV. Kit Contents:

Components	E4391-100	Part No.
Micro ELISA Plate	1 plate	E4391-100-1
Cetuximab Standards (S1 – S7)	1 ml X 7	E4391-100-2.x
Assay Buffer	50 ml	E4391-100-3
Confirmation Reagent	12 ml	E4391-100-4
Peroxidase Conjugate	12 ml	E4391-100-5
TMB substrate (Avoid light)	12 ml	E4391-100-6
Stop Solution	12 ml	E4391-100-7
Wash buffer (20X)	50 ml	E4391-100-8
Plate sealers	2	E4391-100-9

## V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

## VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment.

## VII. Reagent and Sample Preparation:

Note: Prepare reagents within 30 minutes before the experiment.

Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Wash Buffer:** Dilute the 20X Wash Buffer to 1X solution in ddH<sub>2</sub>O (10 ml of Wash Buffer stock to 190 ml of ddH<sub>2</sub>O). Mix the 1X solution thoroughly by vortex manually. The working stock can be stable for 2 weeks after preparation at 4°C.

### 2. Standard Preparation:

Ready to use

Name	S1	S2	S3	S4	S5	S6	S7
Conc. (ng/ml)	500	250	125	62.5	0	High Control	Low Control

### 3. Sample Dilution:

- **Serum/Plasma:** First dilute samples at 1:10 (20 µl Serum/Plasma + 180 µl assay buffer)
- Diluted samples should further be diluted if the concentration of Cetuximab is higher than the measuring range.

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- The usual precautions for venipuncture should be observed. Samples are stable at 4°C for 7 days and -20°C for 6 months. Avoid freeze-and-thaw cycle.
4. **Confirmation Test Mixture:** Mix 210 µl undiluted (positive) serum/plasma sample with 90 µl confirmation reagent in a microtube for 60 minutes prior to the test.

#### VIII. Assay Protocol:

**Note:** Bring all reagents, microplate and samples to room temperature 15 minutes prior to the assay.

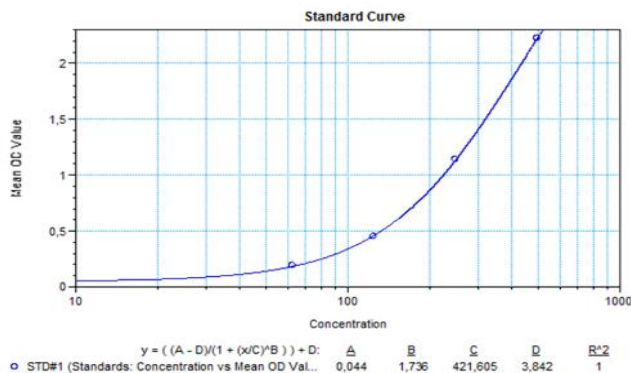
It is recommended that all standards and samples be run at least in duplicate.

A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Add 100 µl of **standards, diluted-samples**, and **confirmation test mixture** (if applicable) into appropriate wells. Cover wells and incubate for 60 minutes at room temperature (RT).
3. Discard incubation solution. Wash plate 3 times each with 300 µl of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
4. Add 100 µl of **Peroxidase Conjugate** into each well. Cover wells with adhesive plate sealer and incubate at RT for 60 minutes.
5. Discard the solution and wash the wells as step 3.
6. Add 100 µl of 1X **TMB substrate** solution and incubate the plate in dark at RT for 20 minutes
7. Add 100 µl of **Stop solution** to stop the reaction
8. Read the absorbance in micro plate reader set to 450 nm within 20 minutes. (reference wavelength to 650 nm)

#### IX. QUANTITATIVE CALCULATION:

Using the standards disregarding zero standard, construct a standard curve by plotting the OD<sub>450/650</sub> nm for each standard on the Y-axis versus the corresponding Cetuximab concentration on the X-axis. Construct a standard curve of difference data using software capable of generating four parameter logistic (4PL) or point-to-point calculation curve fit. To obtain the exact values of the samples, the concentration determined from the standard-curve should be multiplied by the dilution factor.



**Figure:** Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

#### X. QUALITATIVE INTERPRETATION:

- If “Sample OD<sub>450/650</sub> / Zero Standard (S5) OD<sub>450/650</sub>” is < 3, the sample is **NEGATIVE** for Antibody to Cetuximab (ATR)
- If “Sample OD<sub>450/650</sub> /Zero Standard (S5) OD<sub>450/650</sub>” is ≥ 3, the sample is **POSITIVE** for ATR and if required samples may be extrapolated for quantitative analysis and confirmation.
- For the run to be valid, the OD<sub>450/650</sub> nm of Positive Control (Standard A) should be ≥ 1.000 and the OD<sub>450/650</sub> nm of each Negative Control should be <0.200, if not, improper technique or reagent deterioration may be suspected and the run should be repeated.
- Interpretation of true and false positive: For true positive sample, inhibition should be equal or greater than 25%

$$\frac{\text{OD}_{(450/650)} \text{ sample} - \text{OD}_{(450/650)} \text{ sample w/confirmation reagent}}{\text{OD}_{(450/650)} \text{ sample}} \times 100 = \text{inhibition \%}$$

#### XI. RELATED PRODUCTS:

- BioSim™ Rituximab (Mabthera®) (Human) ELISA Kit (Cat. No. E4371-100)
- BioSim™ Adalimumab (Humira®) (Human) ELISA Kit (Cat. No. E4372-100)
- BioSim™ Bevacizumab (Avastin®) (Human) ELISA Kit (Cat. No. E4373-100)
- BioSim™ Etanercept (Enbrel®) (Human) ELISA Kit (Cat. No. E4374-100)
- BioSim™ Infliximab (Remicade®) (Human) ELISA Kit (Cat. No. E4375-100)
- BioSim™ Trastuzumab (Herceptin®) (Human) ELISA Kit (Cat. No. E4376-100)
- BioSim™ Golimumab (Simponi®)(Human) ELISA Kit (Cat. No. E4377-100)
- BioSim™ Infliximab (Remsima®)(Human) ELISA Kit (Cat. No. E4378-100)

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- BioSim™ Cetuximab (Erbix®)(Human) ELISA Kit (Cat. No. E4379-100)
- BioSim™ Denosumab (Prolia®)(Human) ELISA Kit (Cat. No. E4380-100)
- BioSim™ Omalizumab (Xolair®)(Human) ELISA Kit (Cat. No. E4381-100)
- BioSim™ Nivolumab (Opdivo®)(Human) ELISA Kit (Cat. No. E4382-100)
- BioSim™ Pembrolizumab (Keytruda®)(Human) ELISA Kit (Cat. No. E4383-100)
- BioSim™ Ipilimumab (Yervoy®)(Human) ELISA Kit (Cat. No. E4384-100)
- BioSim™ Rituximab (Mabthera®) (Human) ELISA Kit (Cat. No. E4385-100)
- BioSim™ Trastuzumab (Herceptin®) (Human) ELISA Kit (Cat. No. E4386-100)
- BioSim™ Infliximab (Remicade®) (Human) ELISA Kit (Cat. No. E4387-100)
- BioSim™ Adalimumab (Humira®) (Human) ELISA Kit (Cat. No. E4388-100)
- BioSim™ Infliximab (Remsima®) (Human) ELISA Kit (Cat. No. E4390-100)
- BioSim™ Cetuximab (Erbix®) (Human) ELISA Kit (Cat. No. E4391-100)

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