Fas L Eli-pair

❒ **851.730.005 5 plates** ❒ **851.730.010 10 plates**

❒ **851.730.015 15 plates** ❒ **851.730.020 20 plates**

Reagents provided for 5 plates

(Store all Reagents at 4°C)

- Capture Ab B-B34 : 1 vial of 0.5 ml
- Detection biotinylated antibody B-P13 : 1 vial freeze dry
  Reconstitute with 0.55 ml PBS 0.1% Azide w/v or an other preservative.
- 20-- Fas-L Eli-Standard : 5 vials
- Streptavidin-HRP : 1 vial
- Ready-to-use TMB : 2 vials

Reagents and materials not provided

- 96 wells polysterene plate (e.g. Nunc Maxisorp Cat # 468667)
- Coating Buffer : PBS pH 7.2-7.4
- Wash Buffer : PBS containing 0.05% tween 20 v/v
- Saturation Buffer : PBS 5% BSA w/v
- Standard Diluent Buffer : PBS 1%BSA w/v
- Biotinylated Antibody Diluent Buffer : PBS 10%FCSw/v
- HRP-Streptavidin Diluent Buffer : PBS 0.1% tween plus 1% BSA w/v
- PBS 0.1% Azide W/v
- 1M Sulfuric Acid
- Automatic plate washer (Optional)
- ELISA plate reader 450nm

The Fas L Eli-pair includes both the capture and the detection antibodies as well as Horse-Radish peroxidase conjugated Streptavidin (HRP-Strep) and TMB which are the reagents used in the colour development step.
This Eli-pair set is intended to measure Fas L in cell -culture supernatants, as well as in serum samples. The enclosed procedures provide general guidelines for ELISA preparation. This procedure may need some optimization depending on the plastic, matrix, standard range.

These reagents are intended for research use only, not for diagnostic purposes.

ELISA Procedure

Coating and Saturation

. For 1 plate pipette 100µl of the capture antibody into 10 mL of Coating Buffer. Distribute 100µl of the dilution in each well of a polysterene microplate. Cover the plate and incubate overnight at 4°C.
. After 2 washes with approxymately 400µl of PBS-Tween 0.05%, block wells with 250µl of Saturation Buffer for 2 hours at room temperature.
. Empty plate by tapping on absorbant paper and let dry on the bench for 24 hours. The plate is ready to use, and can be stored at 4°C in a dry pouch for at least two weeks.

Method

. Dilute your standards and samples in Standard Diluent Buffer or in the most relevant matrix to your samples (i.e. Serum, Cell-culture medium). For cell-culture medium we recommend the use of standard Diluent Buffer, because biologicals fluids might contain proteases or cytokine-binding proteins that could modify the recognition of the cytokine you want to measure.
. Dispense 100µl of dilution in each well. You should include blank wells (Zero Standard concentration). Remember to run standard and samples in Duplicate. See plate scheme below.
. Cover the plate and incubate 2 hours at room temperature.
. Wash 3 times with approximately 400µl of wash buffer.
. Reconstitute the vial of biotinylated detection anti-Fas L with 0.55 mL of PBS 0.1% Azide. For 1 plate dilute 100 µl of the Detection Antibody into 5 mL of Biotinylated Antibody Diluent Buffer and distribute 50 µl in each well.
. Incubate 1 hour at room temperature.
. Wash 3 times with approximately 400µl of wash buffer and move immediatly to the next step.

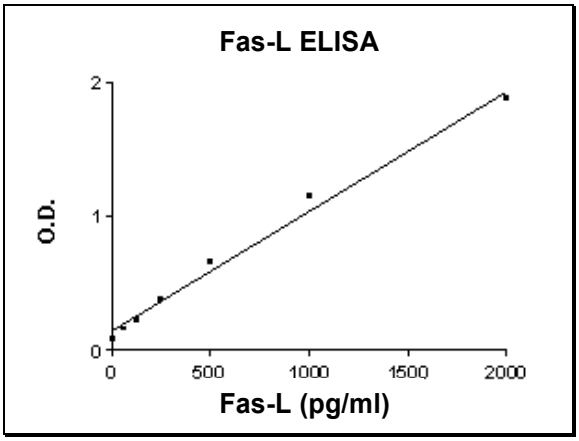
Colour development

. For 1 plate pipette 5µl of HRP-Strep into 500µl of HRP-Streptavidin Diluent Buffer. Take 150µl of this dilution into 10mL of the same buffer. Dispense 100µl per wells. Incubate 20 min at room temperature and wash 3 times with wash buffer.
. Distribute 100µl of ready-to-use TMB and let develop for 10-15 min in the dark (Wrap your plate in aluminium foil).
. Stop the reaction by adding 100µl of 1M H_2SO_4. Read absorbance at 450nm with a reference filter set to 630nm or 650nm.

Analysis

. Average all duplicates (Standards, Samples) optical density (OD) at 450nm.
. Using standard wells values, plot each standard OD (Y axis) versus the corresponding standard concentration (X axis). Draw a standard curve on linear graph paper. For plate reader with automated standard curve calculation capability a linear/Linear curve fit should give the best and most accurate results.
Determine unknown samples concentration from the standard curve based on their OD value. (Example of standard curve is given below). Do not extrapolate the standard curve beyond the 2000 pg/mL point. The dose-response is non-linear in this region and a good accuracy is difficult to obtain. Concentrated samples (i.e. >2000pg/mL) should be diluted with standard diluent buffer. The result obtained should be multiplied by the corresponding dilution factor.

Data Example



Fas L Standard curve ranging from 62.5 to 2000 pg/mL

Suggested Plate Scheme

1 2	3 4	5 6	7 8	9 10	11 12
Blank Wells					
Standard 2000 pg/ml					
Standard 1000 pg/ml					
Standard 500 pg/ml					
Standard 250 pg/ml					
Standard 125 pg/ml					
Standard 62.5 pg/ml					

Wells available for Unknown Samples

Fas L Eli-standard

Eli-standards are lyophilized recombinant proteins to be used with Eli-pair antibody sets. Reconstitution volume and standard range are given as indication and are calculated to be optimal for the corresponding Eli-pair antibody set. **Store lyophilized vials at 4°C.**

Standards should be reconstituted with the volume stated on the vial. This reconstitution produces a stock solution of 2000 pg/mL Fas L. Reconstituted solution should be discarded after use.

Recommended Fas L standard range : 2000 pg/mL- 62.5 pg/mL.

To run the standard curve make a two steps fold dilution ranging from 2000 pg/mL to 62.5 pg/mL. Run each test in **duplicate**. Distribute 100µl of each dilution into wells.
For blank wells dispense 100µl of Standard Diluent Buffer into wells (Zero cytokine concentration).