AXL (Human) ELISA Kit

Catalog Number KA1696

96 assays

Version: 03

Intended for research use only

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Introduction

Principle of the Assay

The AXL (Human) ELISA Kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human Axl in serum, plasma, and cell culture supernatants. This assay employs an antibody specific for human Axl coated on a 96-well plate. Standards and samples are pipetted into the wells and Axl present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human Axl antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Axl bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

General Information

Materials Supplied

Component	Amount
Axl Microplate (Item A): Coated with anti-human Axl.	96 (8x12) wells
Wash Buffer Concentrate (20X) (Item B): 20X concentrated solution.	25 mL
Standard Protein (Item C): Human Axl. 1 vial is enough to run each standard in duplicate.	2 vials
Detection Antibody Axl (Item F): Biotinylated anti-human Axl (each vial is enough to assay half microplate).	2 vials
HRP-Streptavidin Concentrate (Item G): 300x concentrated HRP-conjugated Streptavidin.	200 µL
TMB One-Step Substrate Reagent (Item H): 3, 3', 5, 5'- tetramethylbenzidine (TMB) in buffered solution.	12 mL
Stop Solution (Item I): 0.2 M sulfuric acid.	8 mL
Assay Diluent A (Item D): Contains 0.09% sodium azide as preservative.	30 mL
Assay Diluent B (Item E): 5x concentrated buffer.	15 mL

Storage Instruction

May be stored for up to 6 months at 2º to 8ºC from the date of shipment. Opened Microplate Wells or reagents may be store for up to 1 month at 2º to 8ºC. Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

Reconstituted standard can be stored at -80ºC for up to 1 week.

Note: the kit can be used within one year if the whole kit is stored at -20°C. Avoid repeated freeze-thaw cycles.

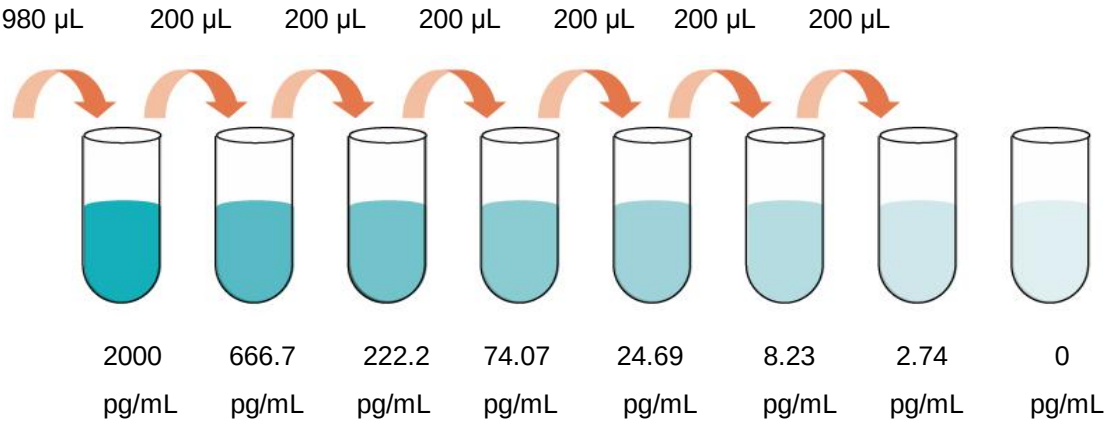
Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Precision pipettes to deliver 2 µL to 1 mL volumes.
- ✓ Adjustable 1-25 mL pipettes for reagent preparation.
- ✓ 100 mL and 1 liter graduated cylinders.
- ✓ Absorbent paper.
- ✓ Distilled or deionized water.
- ✓ Log-log graph paper or computer and software for ELISA data analysis.
- ✓ Tubes to prepare standard or sample dilutions.

Assay Protocol

Reagent Preparation

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water before use.
3. Sample dilution: Assay Diluent A (Item D) should be used for dilution of serum and plasma samples. 1X Assay Diluent B (Item E) should be used for dilution of cell culture supernatant samples. The suggested dilution for normal serum/plasma is 20 - 200 fold.
   Note: Levels of Axl may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.
4. Preparation of standard: Briefly spin a vial of Item C. Add 400 µL Assay Diluent A (for serum/plasma samples) or 1X Assay Diluent B (for cell culture medium) into Item C vial to prepare a 100 ng/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 20 µL Axl standard from the vial of Item C, into a tube with 980 µL Assay Diluent A or 1X Assay Diluent B to prepare a 2,000 pg/mL standard solution. Pipette 400 µL Assay Diluent A or 1X Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1X Assay Diluent B serves as the zero standard (0 pg/mL).



5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1x Wash Buffer.
6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of Assay Procedure.

7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 300-fold with 1x Assay Diluent B (Item E).
   For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 50 µL of HRP-Streptavidin concentrate into a tube with 15 mL 1x Assay Diluent B to prepare a final 300-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µL of each standard (see Reagent Preparation step 4) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.
3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µL of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash as in step 3.
6. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step 3.
8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.

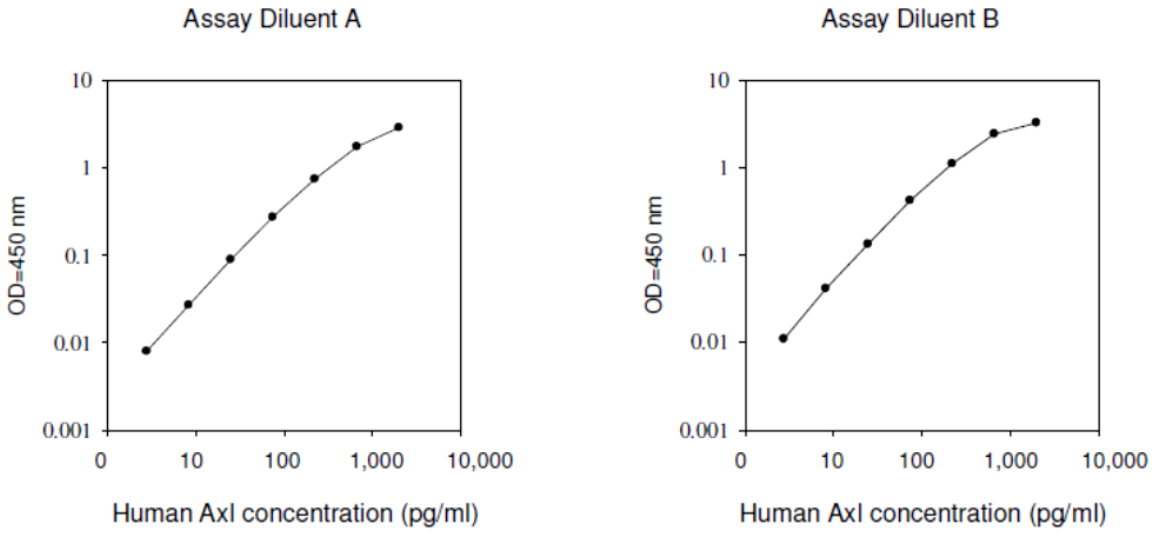
Summary

1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µL standard or sample to each well. Incubate 2.5 hours at room temperature or overnight at 4°C.
3. Add 100 µL prepared biotin antibody to each well. Incubate 1 hour at room temperature.
4. Add 100 µL prepared Streptavidin solution. Incubate 45 minutes at room temperature.
5. Add 100 µL TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
6. Add 50 µL Stop Solution to each well. Read at 450 nm immediately.

Data Analysis

Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.



These standard curves are for demonstration only. A standard curve must be run with each assay.

Performance Characteristics

- Sensitivity

  The minimum detectable dose of Human Axl was determined to be 2 pg/mL.

  Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

- Recovery

  Recovery was determined by spiking various levels of Human Axl into the sample types listed below. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	91.77	82-102
Plasma	93.49	83-103
Cell culture media	92.35	82-102

- Linearity

Sample	Type	Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	93	94	94
	Range (%)	83-103	82-102	84-103
1:4	Average % of Expected	94	92	96
	Range (%)	84-104	83-103	83-103

- Reproducibility

  Intra-Assay: CV<10%

  Inter-Assay: CV<12%

- Specificity

  Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested: human Angiogenin, BDNF, BLC, ENA-78, FGF-4, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, G-CSF, GM-CSF, IFN-γ, Leptin, MCP-1, MCP-2, MCP-3, MDC, MIP-1α, MIP-1 β, MIP-1δ, PARC, PDGF, RANTES, SCF, TARC, TGF-β, TIMP-1, TIMP-2, TNF-α, TNF-β, TPO, VEGF.

Resources

Troubleshooting

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Briefly centrifuge Item C and dissolve the powder thoroughly by gently mixing.
Low signal	Improper preparation of standard and/or biotinylated antibody	Briefly spin down vials before opening. Dissolve the powder thoroughly.
	Too brief incubation times	Ensure sufficient incubation time; assay procedure step 2 may be done overnight.
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation.
Large CV	Inaccurate pipetting	Check pipettes
	Air bubbles in wells	Remove bubbles in wells.
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer.
Low sensitivity	Improper storage of the ELISA kit	Store your standard at <-70°C after reconstitution, others at 4°C. Keep substrate solution protected from light.
	Stop solution	Add stop solution into each well before reading plate.

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H