Human CD117 / c-Kit ELISA Kit

Enzyme Immunoassay for the quantification of Human CD117 / c-Kit in Serum, plasma and cell culture supernatants

Catalog number: ARG81314

Package: 96wells

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INTRODUCTION

This gene encodes the human homolog of the proto-oncogene c-kit. C-kit was first identified as the cellular homolog of the feline sarcoma viral oncogene v-kit. This protein is a type 3 transmembrane receptor for MGF (mast cell growth factor, also known as stem cell factor). Mutations in this gene are associated with gastrointestinal stromal tumors, mast cell disease, acute myelogenous lukemia, and piebaldism. Multiple transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Jul 2008]

Tyrosine-protein kinase that acts as cell-surface receptor for the cytokine KITLG/SCF and plays an essential role in the regulation of cell survival and proliferation, hematopoiesis, stem cell maintenance, gametogenesis, mast cell development, migration and function, and in melanogenesis. In response to KITLG/SCF binding, KIT can activate several signaling pathways. Phosphorylates PIK3R1, PLCG1, SH2B2/APS and CBL. Activates the AKT1 signaling pathway by phosphorylation of PIK3R1, the regulatory subunit of phosphatidylinositol 3-kinase. Activated KIT also transmits signals via GRB2 and activation of RAS, RAF1 and the MAP kinases MAPK1/ERK2 and/or MAPK3/ERK1. Promotes activation of STAT family members STAT1, STAT3, STAT5A and STAT5B. Activation of PLCG1 leads to the production of the cellular signaling molecules diacylglycerol and inositol 1,4,5-trisphosphate. KIT signaling is modulated by protein phosphatases, and by rapid internalization and degradation of the receptor. Activated KIT promotes phosphorylation of the protein phosphatases PTPN6/SHP-1 and PTPRU, and of the transcription factors STAT1, STAT3, STAT5A and STAT5B. Promotes phosphorylation of PIK3R1, CBL, CRK (isoform Crk-II), LYN, MAPK1/ERK2 and/or MAPK3/ERK1, PLCG1, SRC and SHC1. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for CD117 / c-Kit has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any CD117 / c-Kit present is bound by the immobilized antibody. A biotin-conjugated antibody specific for CD117 / c-Kit is then added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of CD117 / c-Kit bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of CD117 / c-Kit in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C
Standard (Lyophilized)	2 vials (10 ng/ml)	4°C
Control (Lyophilized) (7.4 ± 1.9 ng/ml)	2 vials	4°C
10X Standard/Sample diluent	1 X 15 ml	4°C
Antibody conjugate concentrate	1 vial (400 µl)	4°C
Antibody diluent buffer	7 ml (Ready to use)	4°C
HRP-Streptavidin concentrate	2 vials (5 µl)	4°C
HRP-Streptavidin diluent buffer	12 ml (Ready to use)	4°C
200X Wash buffer	10 ml	4°C
TMB substrate	11 ml (Ready to use)	4°C (Protect from light)
STOP solution	11 ml (Ready to use)	4°C
Plate sealer	2 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 610-650 nm as the reference wave length)
- Pipettes and pipette tips
- Deionized or distilled water
- Vortex mixer
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 200X Wash buffer or Standard/Sample diluent concentrate, warm to RT until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Thoroughly mix the reagents and samples before use by agitation or swirling.
- It is highly recommended that the standards and samples be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation (1000 X g, 10 min) and aliquot & store samples at-70°C. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for few minutes before centrifugation for 10 minutes at 1000 x g. Remove serum and

assay immediately or aliquot and store samples at -70°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma on ice using EDTA, citrate or heparin as an anticoagulant. Centrifuge (1000 x g) for 30 minutes at 2-8°C within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-70°C. Avoid repeated freeze-thaw cycles.

Note:

- It is recommended that thaw the samples at room temperature and make sure that sample is completely thawed and homogeneous before use. Do not thaw by heating at 37°C or 56°C.
- Avoid using haemolysed or lipemic samples.
- If large amounts of particles are present these should be removed prior to use by centrifugation or filtration.

REAGENT PREPARATION

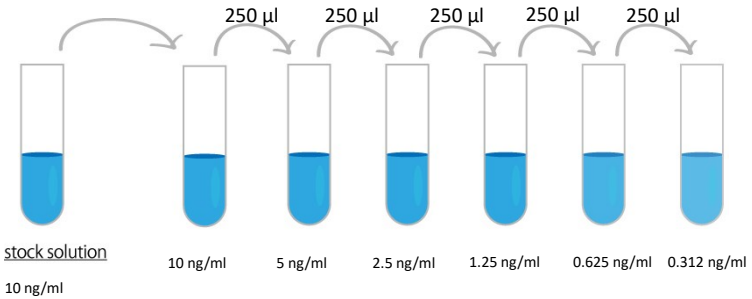
- 1X Wash buffer: Dilute 200X Wash buffer into distilled water to yield 1X Wash buffer (e.g. 10 ml 200X Wash buffer + 1990 ml distilled water). Store the 1X wash buffer at 2-8°C for up to 1 week.
- 1X Antibody conjugate: Prepare immediately before use, dilute 27.5X antibody conjugate concentrate into antibody diluent buffer to yield 1X antibody solution (e.g. for 16 wells, add 40 µl Antibody conjugate concentrate into 1060 µl Antibody diluent buffer). Use 1X Antibody conjugate immediately and do not store.
- HRP-Streptavidin working solution: Prepare immediately before use, dilute 67.67X HRP-Streptavidin concentrate into HRP-Streptavidin diluent buffer to yield 1X HRP-Streptavidin solution (e.g. for 16 wells,

add 30 µl Antibody conjugate concentrate into 2 ml HRP-Streptavidin diluent buffer). Use 1X HRP-Streptavidin solution immediately and do not store.

- **1X Standard/Sample Diluent Buffer:** Dilute 10X Standard/Sample Diluent Buffer into distilled water to yield 1X Standard/Sample Diluent Buffer (e.g. 25 ml Standard/Sample Diluent Buffer + 225 ml distilled water). Store the 1X Standard/Sample Diluent Buffer at 2-8°C for up to 1 week.
- **Control:** Reconstitute the control with 1 ml of 1x Standard/Sample diluent and the concentration will be list on the vial (lot dependent). Allow to stand for 5 mins with gentle swirling before use. Do not store after use.
- **Sample**: Serum or plasmas samples have to be diluted 1:10 in Standard/Sample diluent. If the initial assay found samples contain CD117 / c-Kit higher than the highest standard, the samples can be diluted with Standard/Sample diluent and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

  (It is recommended to do pre-test to determine the suitable dilution factor).
- **Standards**: immediately before use, reconstitute the standard with **(volume depend on the lot)** of 1x Standard/Sample diluent to yield a stock concentration of 10 ng/ml. Mix the reconstituted standard gently by inversion only, and make sure the standard is dissolved completely before making serial dilutions. The 1x Standard/Sample diluent serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted with 250 µl 1x Standard/Sample diluent as according to the

suggested concentration below: 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.312 ng/ml.



ASSAY PROCEDURE

All materials should be equilibrated to room temperature (18 - 25°C) before use, each vial should be mixed thoroughly without foaming prior to use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards, samples, zero standard (Standard/Sample diluent) and control into wells.
3. Add 50 µl 1X Antibody conjugate into each well. Cover wells and incubate for 1 hour at room temperature.

4. Aspirate each well and wash, repeating the process two times for a total

three washes. Wash by filling each well with 1× Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

5. Add 100 µl of 1X HRP-Streptavidin working solution to each well. Cover wells and incubate for 30 minutes at room temperature.
6. Aspirate each well and wash as step 4.
7. Add 100 µl of TMB substrate reagent to each well. Incubate for 10-20 minutes at room temperature in dark.
8. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing.
9. Read the OD with a microplate reader at 450 nm immediately. (optional: read at 610-650 nm as the reference wave length)

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

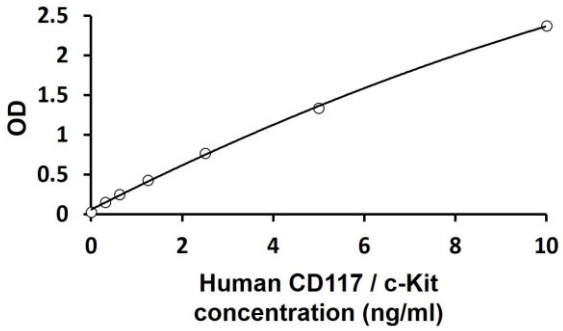
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Human CD117 / c-Kit ranged from 0.312- 10 ng/ml. The mean MDD was 0.095 ng/ml.

Specificity

This assay recognizes natural soluble Human CD117 / c-Kit. No significant cross-reactivity or interference with the factors below was observed:

No cross reactivity with other human soluble receptors

Intra-assay and Inter-assay precision

Intra-assay: 4.8 – 6%.

Inter-assay: 7.6 – 7.9%