ELISA Kit

Catalog # KHO2031 (96 tests)

Human
c-Met (soluble)

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Contents and Storage

Storage

Store at 2 to 8°C.

Contents

Reagents Provided	96 Test Kit
Hu c-Met (soluble) Standard, lyophilized, recombinant Hu c-Met. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials
*Standard Diluent Buffer**. Contains 0.1% sodium azide; red dye*; 60 mL per bottle.	1 bottle
Antibody Coated Wells. 12 x 8 Well Strips.	1 plate
*Hu c-Met (soluble) Biotin Conjugate**, (Biotin-labeled anti-c-Met (soluble). Contains 0.1% sodium azide; blue dye*; 11 mL per bottle.	1 bottle
Streptavidin-HRP (100X). Contains 3.3 mM thymol; 0.125 mL per vial.	1 vial
*HRP Diluent**. Contains 3.3 mM thymol; yellow dye*; 25 mL per bottle.	1 bottle
Wash Buffer Concentrate (25X). 100 mL per bottle.	1 bottle
Stabilized Chromogen, *Tetramethylbenzidine (TMB).* 25 mL per bottle.	1 bottle
Stop Solution. 25 mL per bottle.	1 bottle
Plate Covers, adhesive strips.	4
* In order to help our customers avoid any mistakes in pipetting the ELISAs, we provide colored *Standard Diluent Buffer*, *Detection Antibody*, and *HRP Diluent* to help monitor the addition of solution to the reaction well. This does not in any way interfere with the test results.	

Disposal Note

This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

Safety

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

Introduction

Purpose

The Invitrogen Human c-Met (soluble) ELISA is to be used for the quantitative determination of Hu c-Met (soluble) in serum, plasma, and tissue culture medium. The assay will recognize both natural and recombinant Hu c-Met (soluble).

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

Principle of the Method

The Invitrogen Hu c-Met (soluble) kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A highly purified antibody has been coated onto the wells of the microtiter strips provided.

During the first incubation, standards of known Hu c-Met (soluble) content, controls, and unknown samples are pipetted into the wells. After washing, biotinylated secondary antibody, is added.

After washing, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a third incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of Hu c-Met (soluble) present in the original specimen.

Background Information

c-Met, a member of the tyrosine kinase superfamily, is the receptor for hepatocyte growth factor, also known as scatter factor (HGF/SF). The mature c-Met protein is a disulfide-linked heterodimer with M_r=190 kDa composed of a heavily glycosylated extracellular α subunit, and a β subunit comprised of an extracellular ligand binding domain, a single transmembrane domain, and a cytoplasmic tyrosine kinase domain. c-Met is transcribed from a single open reading frame and translated into a protein precursor that is proteolytically cleaved, yielding the heterodimeric mature protein. Alternative splicing yields several c-Met isoforms, including an uncleaved, monomeric form or isoforms lacking regions of the cytoplasmic kinase domain. Cells expressing c-Met include epithelial cells, endothelial cells, blood cells of various types, and glomerular mesenchymal cells.

The ligand for c-Met, HGF/SF, is a member of the plasminogen-related growth factor family, which is synthesized as an inactive pro-form. HGF/SF activation requires cleavage with either urokinase plasminogen activator (uPA), HGF activator, or Coagulation Factor Xa. Sources of HGF/SF include mesenchymal cells, mesanglial cells, endothelial cells, macrophages, and tumor cells.

HGF/SF binding to c-Met stimulates receptor dimerization and the phosphorylation of numerous residues within the receptor's cytoplasmic domain, including tyrosines 1230, 1234, and 1235 within the Tyr-X-X-X-Tyr-Tyr motif of c-Met's activation loop. This motif is conserved among the activation loops of several receptor tyrosine kinases including insulin receptor, insulin-like growth factor 1 receptor, nerve growth factor receptor/Trks, and RON. Phosphorylation of tyrosines 1234 and 1235 of c-Met is required for activation of the receptor's tyrosine kinase activity. c-Met phosphorylation also generates docking sites for numerous signaling molecules and stimulates receptor internalization via clathrin-coated vesicles. Signaling proteins that are phosphorylated and/or localized in response to c-Met phosphorylation include: Grb2, Shc, Cbl, Crk, cortactin, paxillin, GAB1, PI-3 K, FAK, Src, Ras, ERK1 and 2, JNK, PLC-γ, AKT, and STAT3.

HGF/SF stimulation of c-Met expressing cells enhances proliferation, migration, morphogenesis, and protease synthesis, characteristics that are associated with invasive cell phenotype.

Soluble c-Met is a truncated form of the c-Met membrane receptor. It can be cleaved by proteases and released from the lipid bilayer in a process known as ectodomain shedding. Many transmembrane proteins are released through this shedding process and it is a normal part of development which when defective can cause a number of pathologies. The soluble form of the c-Met receptor is smaller than the membrane bound receptor, contains the extracellular region of the receptor, and is able to bind the HGF ligand.

Methods

Materials Needed But Not Provided

- Microtiter plate reader (at or near 450 nm) with software
- Calibrated adjustable precision pipettes
- Distilled or deionized water
- Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.)
- Glass or plastic tubes for diluting solutions
- Absorbent paper towels
- Calibrated beakers and graduated cylinders

Procedural Notes

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin-free tubes.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all standards, controls and samples be run in duplicate.
7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
8. **Do not mix or interchange different reagent lots from various kit lots**.
9. Do not use reagents after the kit expiration date.
10. Absorbances should be read immediately, but can be read up to 2 hours after assay completion. For best results, keep plate covered in the dark.
11. In-house controls or kit controls, if provided, should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
12. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.
13. Because Stabilized *Chromogen* is light sensitive, avoid prolonged exposure to light. Avoid contact between chromogen and metal, or color may develop.

Directions for Washing

- **Incomplete washing will adversely affect the test outcome**. All washing must be performed with the *Wash Buffer Concentrate (25X)* provided.
- Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip into the bottom of each well. Take care not to scratch the inside of the well. After aspiration, fill the wells with at least 0.4 ml of diluted *Wash Buffer*. Let soak for 15 to 30 seconds, and then aspirate the liquid. Repeat as directed under Assay Procedure. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
- Alternatively, the diluted *Wash Buffer* may be put into a squirt bottle. If a squirt bottle is used, flood the plate with the diluted *Wash Buffer*, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
- If using an automated washer, follow the washing instructions carefully.

Preparation of Reagents

Sample Preparation

Human serum and plasma require a 100-fold dilution in the *Standard Diluent Buffer*. For these samples, add 5 µl of the sample to a clean microfuge tube, followed by 495 µl of *Standard Diluent Buffer*. Mix well by gentle pipetting or inversion. Cell culture samples must be diluted *at least 2-fold* (for example by adding 120 µl of sample into 120 µl of buffer) While a 1:2 dilution has been found to work for many cell lines, some cell supernatants may require dilutions up to 1:100.

Dilution of Standard

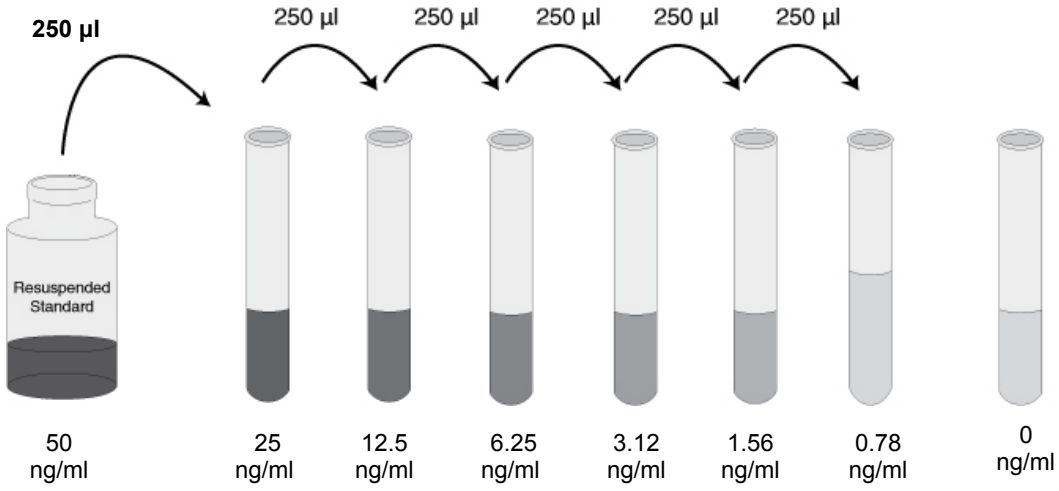
Note: Either glass or plastic tubes may be used for standard dilutions.

The *Hu c-Met (soluble) Standard* is prepared from a highly purified mouse myeloma-expressed recombinant protein.

1. Reconstitute standard to 50 ng/ml with *Standard Diluent Buffer*. Refer to the standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Label as 50 ng/ml Hu c-Met (soluble). Use the standard within 15 minutes of reconstitution.
2. Add 0.250 ml of *Standard Diluent Buffer* to each of 6 tubes labeled 25, 12.5, 6.25, 3.12, 1.56, and 0.78 ng/ml Hu c-Met (soluble).
3. Make serial dilutions of the standard as described in the following dilution diagram. Mix thoroughly between steps.

Note

Remaining reconstituted standard should be discarded. Return the *Standard Diluent Buffer* to the refrigerator.



Preparing SAV-HRP

Note: Prepare within 15 minutes of usage. The *Streptavidin-HRP (100X)* is in 50% glycerol, which is viscous. To ensure accurate dilution, allow *Streptavidin-HRP (100X)* to reach room temperature. Gently mix. Pipette *Streptavidin-HRP (100X)* slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

1. Dilute 10 µl of this 100X concentrated solution with 1 mL of *HRP Diluent* for each 8-well strip used in the assay. Label as Streptavidin-HRP Working Solution.
2. Return the unused *Streptavidin-HRP (100X)* to the refrigerator.

# of 8-Well Strips	Volume of Streptavidin-HRP (100X)	Volume of Diluent
2	20 µl solution	2 ml
4	40 µl solution	4 ml
6	60 µl solution	6 ml
8	80 µl solution	8 ml
10	100 µl solution	10 ml
12	120 µl solution	12 ml

Dilution of Wash Buffer

1. Allow the *Wash Buffer Concentrate (25X)* to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the *Wash Buffer Concentrate (25X)* with 24 volumes of deionized water (e.g., 50 ml may be diluted up to 1.25 liters, 100 ml may be diluted up to 2.5 liters). Label as Working Wash Buffer.
2. Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

Assay Procedure

Be sure to read the Procedural Notes section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Dilute serum and plasma samples 1:100 with *Standard Diluent Buffer*. (See **Preparation of Reagents**.) Cell culture sample must be diluted at least 1:2.
3. Add 100 µl of the *Standard Diluent Buffer* to the zero standard wells. Well(s) reserved for chromogen blank should be left empty.
4. Add 100 µl of standards, controls, or samples (serum, plasma or cell culture prediluted) to the appropriate microtiter wells.
5. Cover plate with *plate cover* and incubate for **2 hours at room temperature**.
6. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing.**
7. Add 100 µl of biotinylated *Hu c-Met (soluble) Biotin Conjugate* solution into each well except the chromogen blank(s). Tap on the side of the plate for 30 seconds to mix.
8. Cover plate with plate cover and incubate for **1 hour at room temperature**.
9. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing.**
10. Add 100 µl Streptavidin-HRP Working Solution to each well except the chromogen blank(s). See **Preparation of Reagents.**

11. Cover plate with the plate cover and incubate for **30 minutes at room temperature.**
12. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing**.
13. Add 100 µl of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
14. Incubate for **30 minutes at room temperature and in the dark. Note: Do not cover the plate with aluminum foil or metalized mylar**. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceeds the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.
15. Add 100 µl of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
16. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µl each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 30 minutes after adding the *Stop Solution*.
17. Use a curve fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit.
18. Read the concentrations for unknown samples and controls from the standard curve. **Multiply value(s) obtained for serum and plasma by 100 and multiply values obtained for cell culture supernatants by at least 2 to correct for the dilution of the samples in step 5**. Samples producing signals greater than that of the highest standard should be further diluted in the *Standard Diluent Buffer* and reanalyzed. Multiply the concentration found by the appropriate dilution factor.

Typical Data (Example)

The following data were obtained for the various standards over the range of 0 to 50 ng/ml Hu c-Met (soluble).

Standard Hu c-Met (soluble) (ng/ml)	Optical Density (450 nm)
50	2.807
25	1.812
12.5	1.132
6.25	0.701
3.12	0.447
1.56	0.266
0.78	0.182
0	0.083

Performance Characteristics

Sensitivity

The minimum detectable dose of Hu c-Met (soluble) is < 0.5 ng/ml. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times, and calculating the corresponding concentration.

Precision

1. Intra-Assay Precision

Samples of known Hu c-Met (soluble) concentration were assayed in replicates of 12 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/ml)	20.27	9.57	4.53
SD	1.23	0.64	0.25
%CV	6.04	6.64	5.56
SD = Standard Deviation CV = Coefficient of Variation			

2. Inter-Assay Precision

Samples were assayed 36 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2	Sample 3
Mean (pg/ml)	19.50	9.74	4.70
SD	1.80	0.79	0.33
%CV	9.22	8.13	7.00
SD = Standard Deviation CV = Coefficient of Variation			

Linearity of Dilution

GTL16 cells were grown in tissue culture media containing 10% fetal bovine serum. 24 hours after changing the media, the cell culture supernatant was harvested. The GTL16 cell culture supernatant was diluted in *Standard Diluent Buffer* over the range of the assay and measured for c-Met (Soluble) content. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

	Cell Culture		
Dilution	**Measured (ng/ml)**	**Expected (ng/ml)**	**% Expected**
1/20	47.80	47.80	100
1/40	20.33	23.90	85.1
1/80	11.10	11.95	92.1
1/160	6.21	5.98	104
1/320	2.49	2.99	83.5

Recovery

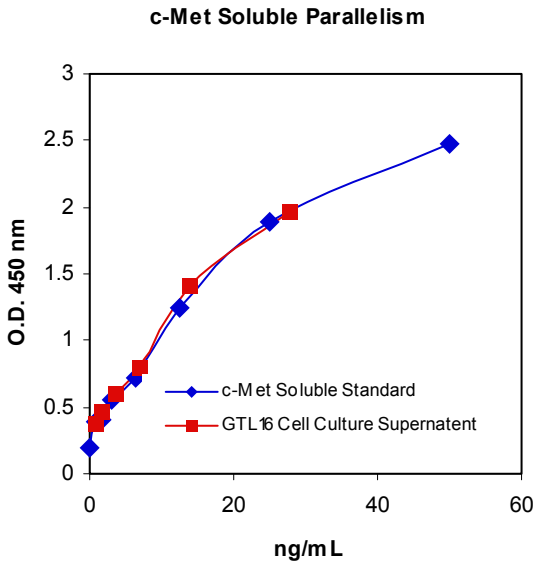
The recovery of recombinant Hu c-Met (soluble) added to tissue culture medium containing 10% fetal bovine serum were measured on the Invitrogen Hu c-Met (soluble) ELISA kit.

Sample Type	Average % Recovery
DMEM+10% calf serum	91

Spike and recovery tests were not performed for serum and plasma due to the high endogenous levels of c-met (soluble). At the 1:100 sample dilution for serum and plasma, recovery assessment would not be valid.

Parallelism

Natural c-Met (soluble) from GTL16 cell culture supernatant was serially diluted in *Standard Diluent Buffer.* The optical density of each dilution was plotted against the c-Met (soluble) standard curve. Parallelism was demonstrated by the figure below and indicated that the standard accurately reflects human c-Met (soluble) content in samples.



Specificity

Buffered solutions of a panel of substances ranging in concentrations from 4,000 to 50,000 pg/ml were assayed with the Invitrogen Human c-Met (soluble) ELISA kit and found to have no cross-reactivity: Human Eotaxin, GM-CSF, IFN-α, IFN-γ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, IP-10, MCP-1, MIG, MIP-1α, MIP-1β, RANTES, sICAM-1, TNF-α, TRAIL, VEGF; Mouse FGFb, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17, IP-10, KC, MCP-1, MIG, MIP-1α, TNF-α, VEGF; and Rat GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-6, IL-10, IL-12, IL-13, TNF-α, MCP-1, MIP-2, RANTES, EGF, HGF, FGFb, VEGF, PDGFRM, VEGFR.

Random, normal serum, plasma and cell culture samples from mouse and rat were also evaluated with the Hu c-Met (soluble) ELISA kit. No cross-reactivity was observed with either mouse or rat samples.

Expected Values

The Hu c-Met (soluble) ELISA kit can be used to measure the levels of human c-Met (soluble) in serum, plasma, and in several different cell lines. Random normal human serum and plasma samples were diluted 1:100 and evaluated with the Invitrogen Hu c-Met (soluble) ELISA kit. GTL16, Huvec, CCD1070SK, HT1080, Jar, and MCF-7 cell lines were cultured in tissue culture media with 10% fetal bovine serum. Media was harvested 24 hours after being added to the cells, was spun down in a centrifuge, and then the supernatant was analyzed using the Hu c-Met (soluble) ELISA. All samples were diluted 1:2 except for GTL16 which was diluted 1:100.

Sample	Range (ng/ml)	Average (ng/ml)
Serum (n=4)	1094-1821	1348
Plasma (n=3)	820-1319	1036
GTL16 (n=1)	-	1562
Huvec (n=2)	11.8-12.8	12.3
CCD1070SK (n=3)	1.71-3.99	2.75
HT1080 (n=2)	113-120	116
Jar (n=1)	-	6.08
MCF-7 (n=2)	0-0.53	0.26

Limitations of the Procedure

Do not extrapolate the standard curve beyond the top standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute all samples above the top standard point with *Standard Diluent Buffer*; reanalyze these and multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of degradation of native Hu c-Met (soluble) in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

Appendix

Troubleshooting Guide

Elevated background	*Cause:* Insufficient washing and/or draining of wells after washing. Solution containing either biotin or SAV-HRP can elevate the background if residual is left in the well. *Solution:* Wash according to the protocol. Verify the function of automated plate washer. At the end of each washing step, invert plate on absorbent tissue on countertop and allow to completely drain, tapping forcefully if necessary to remove residual fluid. *Cause:* Contamination of substrate solution with metal ions or oxidizing reagents. *Solution:* Use distilled/deionized water for dilution of wash buffer and use plastic equipment. DO NOT COVER plate with foil. *Cause:* Contamination of pipette, dispensing reservoir or substrate solution with SAV-HRP conjugate. *Solution:* Do not use chromogen that appears blue prior to dispensing onto the plate. Obtain new vial of chromogen. *Cause:* Incubation time is too long or incubation temperature is too high. *Solution:* Reduce incubation time and/or temperature.
Elevated sample/ standard ODs	*Cause:* Incorrect dilution of standard stock solution; intermediary dilutions not followed correctly. *Solution*: Follow the protocol instructions regarding the dilution of the standard. *Cause:* Incorrect dilution of the Streptavidin-HRP Working Solution. *Solution:* Warm solution of Streptavidin-HRP (100X) to room temperature, draw up slowly and wipe tip with kim-wipe to remove excess. Dilute ONLY in HRP diluent provided. *Cause*: Incubation times extended. *Solution:* Follow incubation times outlined in protocol. *Cause:* Incubations carried out at 37°C when RT is dictated. *Solution:* Perform incubations at RT (= 25 ± 2°C) when instructed in the protocol.
Poor standard curve	*Cause:* Improper preparation of standard stock solution. *Solution:* Dilute lyophilized standard as directed by the vial label only with the standard diluent buffer or in a diluent that most closely matches the matrix of your sample. *Cause:* Reagents (lyophilized standard, standard diluent buffer, etc.) from different kits, either different cytokine or different lot number, were substituted. *Solution:* NEVER substitute any components from another kit. *Cause*: Errors in pipetting the standard or subsequent steps. *Solution*: Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device.

Weak/no color develops	*Cause:* Reagents not at RT (25 ± 2°C) at start of assay. *Solution:* Allow ALL reagents to warm to RT prior to commencing assay. *Cause:* Incorrect storage of components, e.g., not stored at 2 to 8°C. *Solution:* Store all components exactly as directed in protocol and on labels. *Cause:* Working Streptavidin-HRP solution made up longer than 15 minutes before use in assay. *Solution:* Use the diluted Streptavidin-HRP within 15 minutes of dilution. *Cause:* TMB solution lost activity. *Solution 1:* The TMB solution should be clear before it is dispensed into the wells of the microtiter plate. An intense aqua blue color indicates that the product is contaminated. Please contact Technical Support if this problem is noted. To avoid contamination, we recommend that the quantity required for an assay be dispensed into a disposable trough for pipetting. Any TMB solution left in the trough should be discarded. *Solution 2:* Avoid contact of the TMB solution with items containing metal ions. *Cause:* Attempt to measure analyte in a matrix for which the ELISA assay has not been optimized. *Solution:* Please contact Technical Support for advice when using nonvalidated sample types. *Cause:* Wells have been scratched with pipette tip or washing tips. *Solution:* Use caution when dispensing and aspirating into and out of microwells.
Poor Precision	*Cause:* Errors in pipetting the standards, samples or subsequent steps. *Solution:* Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device. Check for any leaks in the pipette tip. *Cause:* Repetitive use of tips for several samples or different reagents. *Solution:* Use fresh tips for each sample or reagent transfer. *Cause:* Wells have been scratched with pipette tip or washing tips. *Solution:* Use caution when dispensing and aspirating into and out of microwells.

Technical Support

Contact Us

For more troubleshooting tips, information, or assistance, please call, email, or go online to www.invitrogen.com/ELISA.

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Limited Warranty

Licensing Information

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Explanation of symbols

Symbol	Description	Symbol	Description
	Catalogue Number		Batch code
	Research Use Only		*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer		European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

Human c-Met (soluble) Assay Summary

Add 100 µL of standards/controls/serum/ plasma/Tissue Culture Supernatant (TCS) (Dilute serum/plasma 1:100 and TCS ≥ 1:2)

Incubate for 2 hr at RT

aspirate and wash 4x

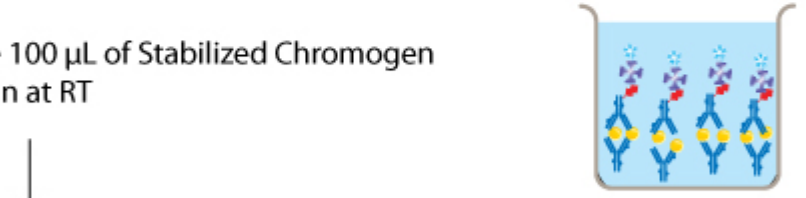
Add 100 µL of Biotin Conjugate
Incubate for 1 hr at RT

aspirate and wash 4x

Incubate 100 µL of Streptavidin-HRP Working Solution for 30 min at RT

aspirate and wash 5x

Incubate 100 µL of Stabilized Chromogen for 30 min at RT



Add 100 µL of Stop Solution
Read at 450 nm

Total time: 4 hr