Human c-Met (soluble) ELISA Kit

Catalog Number KHO2031 (96 tests)

Pub. No. MAN0014703 **Rev.** A.0 (31)

CAUTION! 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.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ Human c-Met (soluble) ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of human c-Met (soluble) in serum, plasma, and cell culture medium. The assay will recognize both natural and recombinant human c-Met (soluble).

c-Met, a member of the tyrosine kinase superfamily, is the receptor for hepatocyte growth factor, also known as scatter factor (HGF/SF). c-Met isoforms, include 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. Sources of HGF/SF include mesenchymal cells, mesanglial cells, endothelial cells, macrophages, and tumor cells.

Soluble c-Met is a truncated form of the c-Met membrane receptor.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KHO2031 (96 tests)
Hu c-Met (soluble) Standard, lyophilized; contains 0.1% sodium azide.	2 vials
Standard Diluent Buffer; contains 0.1% sodium azide and red dye[1]	60 mL
Antibody Coated Plate, 96-well plate	1 plate
Hu c-Met (soluble) Biotin Conjugate; contains 0.1% sodium azide and blue dye[1]	11 mL
Streptavidin-HRP (100X)	0.15 mL
HRP Diluent; contains 3.3 mM thymol and yellow dye[1]	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Plate Covers, adhesive strips	4

[1] 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.

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Pre-dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

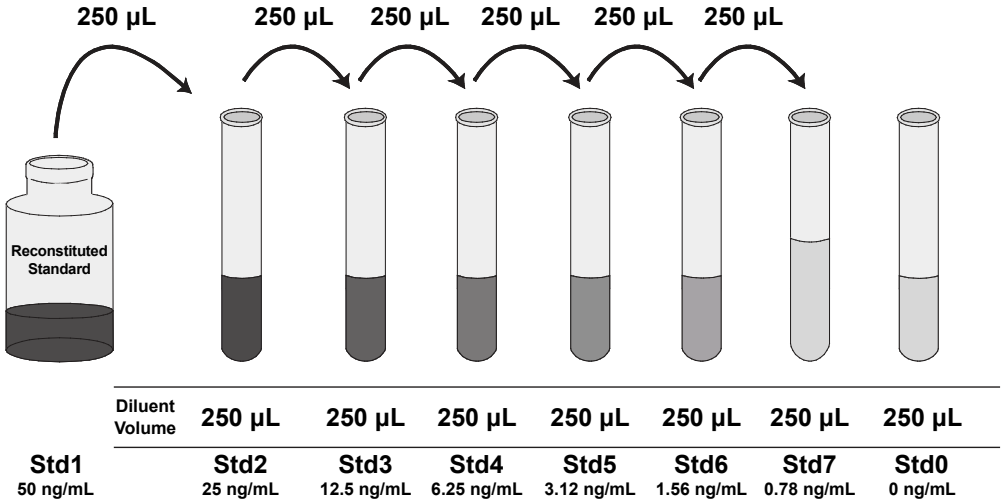
- Perform sample dilutions with Standard Diluent Buffer.
- Dilute human serum or plasma samples 1:100 in Standard Diluent Buffer (e.g., add 5 µL sample into 495 µL buffer).
- Dilute cell culture samples at least 1:2 in Standard Diluent Buffer. For example, add 120 µL of sample into 120 µL of buffer (e.g., 120 µL sample into 120 µL buffer).

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

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

1. Reconstitute Hu c-Met (soluble) Standard to 50 ng/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 50 ng/mL human c-Met (soluble). **Use the standard within 15 minutes of reconstitution.**
2. Add 250 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 25, 12.5, 6.25, 3.12, 1.56, 0.78, and 0 ng/mL human c-Met (soluble).
3. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
4. Remaining reconstituted standard should be discarded or frozen in aliquots at -80°C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.



Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

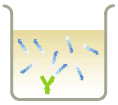
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 4 hours)

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 Bind antigen



a. Add 100 µL of standards, controls, or samples (see "Pre-dilute samples" on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
b. Cover the plate with a plate cover and incubate for 2 hours at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Biotin Conjugate

a. Add 100 µL Hu c-Met (soluble) Biotin Conjugate solution into each well except the chromogen blanks.
b. Cover the plate with plate cover and incubate for 1 hour at room temperature .
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

3 Add Streptavidin-HRP

a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

4 Add Stabilized Chromogen

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

5 Add Stop Solution

Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 30 minutes after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve example

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

Standard Human c-Met (soluble) (ng/mL)	Optical Density (450 nm)
50	2.81
25	1.81
12.5	1.13
6.25	0.70
3.12	0.45
1.56	0.27
0.78	0.18
0	0.08

Inter-assay precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	19.50	9.74	4.70
Standard Deviation	1.80	0.79	0.33
% Coefficient of Variation	9.22	8.13	7.00

Intra-assay precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	20.27	9.57	4.53
Standard Deviation	1.23	0.64	0.25
% Coefficient of Variation	6.04	6.64	5.56

Expected values

The Human 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 Human c-Met (soluble) ELISA Kit. GTL16, Huvec, CCD1070SK, HT1080, Jar, and MCF-7 cell lines were cultured in cell 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 Human c-Met (soluble) ELISA Kit. All samples were diluted 1:2 except for GTL16 which was diluted 1:100.

Sample	Range (ng/mL)	Average (ng/mL)
Serum (n=4)	1,094 to 1,821	1,348
Plasma (n=3)	820 to 1,319	1,036
GTL16 (n=1)	—	1,562
Huvec (n=2)	11.8 to 12.8	12.3
CCD1070SK (n=3)	1.71 to 3.99	2.75
HT1080 (n=2)	113 to 120	116
Jar (n=1)	—	6.08
MCF-7 (n=2)	0 to 0.53	0.26

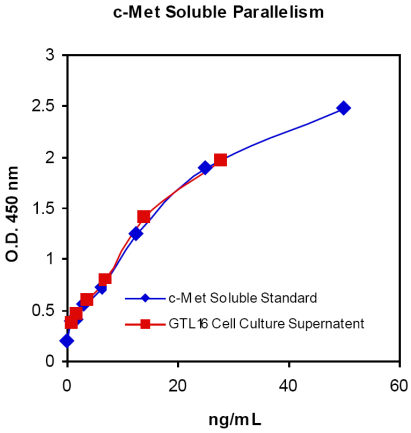
Linearity of dilution

GTL16 cells were grown in cell 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 human c-Met (soluble) content. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

Dilution	Cell Culture		
	Measured (ng/mL)	Expected	
		(ng/mL)	%
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

Parallelism

Natural human 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 human c-Met (soluble) standard curve. The standard accurately reflects human c-Met (soluble) content in samples.



Recovery

The recovery of recombinant human c-Met (soluble) added to cell culture medium containing 10% fetal bovine serum were measured on the Human c-Met (soluble) ELISA Kit.

Sample	Average % Recovery
DMEM+10% calf serum	91

Sensitivity

The analytical sensitivity of the assay is <0.5 ng/mL human c-Met (soluble). This was determined by adding two standard deviations to the mean O.D. obtained from 30 assays of the zero standard.

Specificity

Buffered solutions of a panel of substances ranging in concentrations from 4,000 to 50,000 ng/mL were assayed with the 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.

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

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

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

The information in this guide is subject to change without notice.