c-MET (Soluble) Antibody Pair
5 Plate Format
Lot-specific Technical Data Sheet

Catalog #: CHO0315 Lot #: * A11893

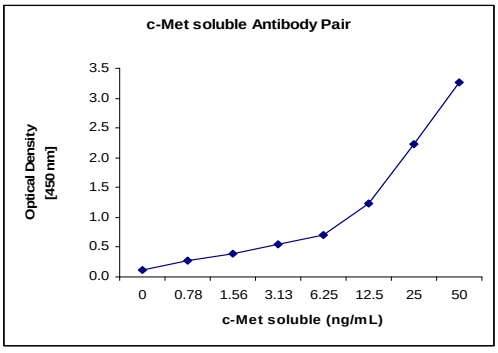
*Note: A letter at the end of the lot number signifies an additional packaging of this same lot.

Intended Use and Materials Provided

The Antibody Pair for c-MET soluble contains components required to construct an enzyme-linked immunoassay for the specific and quantitative measurement of c-MET soluble. Sufficient quantities of all reagents are provided to yield 5 plates of 96 wells if the recommended assay procedure and recommended storage and handling of materials are followed as specified on this insert.

1.	**Coating Antibody:**	**Anti-c-MET soluble Coating Antibody (0.2 mL)**
	Part Number:	CAHO031
	Lot Number:	A11884
	Form:	Liquid, 1 vial, contains 0.1% sodium azide
	Storage:	Store at 2 to 8ºC until expiration date.
	Recommended Dilution:	Dilute to 1:250 with Coating Buffer B (Cat. # CB01100, or see Recommended Buffers). For example, to make 10 mL (enough to coat 1 plate), add 40 µL coating antibody to 9.960 mL Coating Buffer B.
2.	**Detection Antibody:**	**Anti-c-MET soluble Detection Antibody (0.2 mL)**
	Part Number:	DAHO031
	Lot Number:	A11885
	Form:	Liquid, 1 vial, contains 0.1% sodium azide
	Storage:	Store at 2 to 8ºC until expiration date.
	Recommended Dilution:	Dilute to 1:250 with Assay Buffer (Cat. # DS98200, or see Recommended Buffers). For example, to make enough for 1 plate, add 40 µL detection antibody to 9.960 mL Assay Buffer.
3.	**Standard:**	**Recombinant c-MET soluble Standard**
	Part Number:	SD251 (additional vials of standard may be purchased using this part number)
	Lot Number:	A11851
	Form:	Lyophilized, 3 vials
	Storage:	Store at 2 to 8ºC.
	Reconstitution:	Reconstitute with Assay Buffer (Cat. # DS98200 or see Recommended Buffers) according to instructions on vial to yield a stock of 50 ng/mL. Use the standard stock immediately or aliquot into polypropylene tubes and freeze at –80ºC. *Do not store at room temperature or at 4ºC and do not subject to more than one freeze-thaw cycle.*
	Standard Curve:	Add 300 µL Assay Buffer to 6 tubes and label as 25, 12.5, 6.25, 3.13, 1.56 and 0.78 ng/mL. Make serial dilutions starting with 50 ng/mL by transferring 300 µL of each standard to next tube pipetting up and down for each tube. Assay Buffer should be used as the zero standard.
4.	**Streptavidin-HRP:**	**0.2 mL**
	Part Number:	SNN4004Y
	Lot Number:	A11876
	Form:	Liquid, 1 vial, contains 0.05% thymol
	Storage:	Store at 2 to 8ºC until expiration date.
	Recommended Dilution:	Dilute to 1:1000 with Assay Buffer. For example, to make enough for 1 plate, add 10 µL of streptavidin-HRP to 9.990 mL of Assay Buffer (Cat. # DS98200 or see Recommended Buffers).

Representative standard curve was generated by following the recommended assay procedure, which includes the use of the **Invitrogen Antibody Pair Buffer Set (Cat. # CNB0011)**



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Additional Materials Required

- 96 well NUNC MaxiSorp microplates; NUNC Cat. # 434797.
- Pipettes and timer.
- Microplate reader with a detector that can measure absorbance at 450 nm.
- 1 L graduated cylinder; plate washer or wash bottle.
- Polypropylene tubes for standards and sample dilutions, if needed.

Recommended Buffers and Solutions

The Invitrogen Antibody Pair Buffer Set (Cat. # CNB0011) containing Coating Buffers A and B, Assay Buffer, Substrate Solution (TMB), Stop Solution, and Wash Buffer is recommended.

1. **Coating Buffer A:** ***Coating Buffer A (Cat. # CB07100) from Invitrogen is recommended.*** *Alternate buffer choice listed below.*
   8.0 g NaCl, 1.13 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, 0.1% ProClin™; q.s. to 1.0 L with distilled H_2O, pH to 7.4.
2. **Coating Buffer B:** ***Coating Buffer B (Cat. # CB01100) from Invitrogen is recommended.*** *Alternate buffer choice listed below.*
   4.3 g $NaHCO_3$, 5.3 g Na_2CO_3, 0.1% ProClin™, q.s. to 1.0 L with distilled H_2O, pH to 9.4.
3. **Assay Buffer:** ***Assay Buffer (Cat. # DS98200) from Invitrogen is recommended.*** *Alternate buffer choice listed below.*
   8.0 g NaCl, 1.13 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, 5.0 g bovine serum albumin (fraction V), 1 mL Tween 20 and 0.5% ProClin™ as a preservative; q.s. to 1.0 L with distilled H_2O, pH to 7.4.
4. **Wash Buffer:** ***Wash Buffer 25x (Cat. # WB01) from Invitrogen is recommended.*** *Alternate buffer choice listed below.*
   0.2 g KH_2PO_4, 1.9 g K_2HPO_4-$3H_2O$, 0.4 g EDTA, 0.5 mL Tween 20; q.s. to 1.0 L with distilled H_2O, pH to 7.4.
5. **Substrate Solution:** ***TMB (Cat. # SB01) from Invitrogen is recommended.*** *Alternate solution choice listed below.*
   Tetramethylbenzidine (TMB) and Hydrogen Peroxide.
6. **Stop Solution:** ***Stop Solution (Cat.# SS01100) from Invitrogen is recommended.*** *Alternate solution choice listed below.*
   1.8 N H_2SO_4.

Assay Optimization

Antibody Pairs from Invitrogen are designed to be very flexible for your experiments. Consequently, the assay procedure contains only recommendations. The assay procedure has been optimized for use with tissue culture samples. However, serum and plasma samples may be used but may require that certain assay parameters be modified. Investigators are advised to determine optimal buffer formulations, concentrations and incubation times for individual applications.

Recommended Assay Procedure

1. Prepare coating solution by diluting the coating antibody. See "coating antibody" section for the recommended coating antibody dilution.
2. Coat plates with 100 µL per well of the coating solution. Cover plates and incubate overnight (12-18 hr.) at 4ºC.
3. Aspirate wells and wash 1 time with ≥ 400 µL of Wash Buffer per well. Following wash, invert and tap on absorbent paper to remove excess liquid.
4. Block plate with 200 µL per well of Assay Buffer for 1 hour at room temperature.
5. Aspirate, invert, and tap on absorbent paper to remove excess liquid.
6. Prepare standards and sample dilutions in Assay Buffer (or in a diluent that most closely matches the matrix of your sample). For recommended dilutions and storage of the standard, see "standard" section.
7. Pipette 100 µL of standards (in duplicate) and samples into designated wells. ***Incubate for 2 hours at room temperature on the shaker.***
8. Aspirate and wash 5 times using the method in step 3.
9. Add 100 µL of the working detection antibody into each well. For recommended dilutions, see "detection antibody" section. ***Incubate for 1 hour at room temperature.***
10. Aspirate and wash 5 times using the method in step 3.
11. Add 100 µL of the working streptavidin-HRP solution into each well. For recommended dilutions, see "streptavidin-HRP conjugate" section. ***Incubate for 30 minutes at room temperature.***
12. Aspirate and wash 5 times using the method in step 3.
13. Add 100 µL of the TMB substrate to each well. ***Incubate for 30 minutes at room temperature***.
14. Add 100 µL of Stop Solution to each well.
15. Measure absorbance at 450 nm (reference absorbance: 650 nm) within 30 minutes of adding Stop Solution. Calculate results using a log-log or 4-parameter curve fit.