Human VEGF Antibody Pair Kit

10 Plate Format

Lot-specific Technical Data Sheet

Catalog # CHG0113

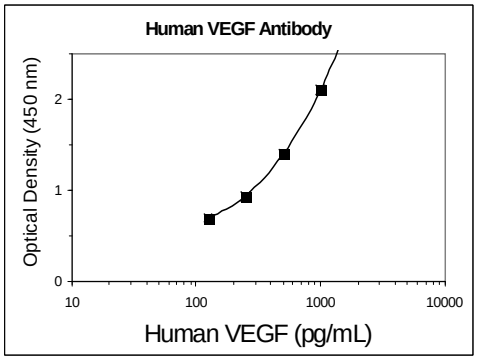
Lot #*: 650073

Intended Use and Materials Provided

The Antibody Pair Kit for Human VEGF contains components required to construct an enzyme-linked immunoassay for the specific and quantitative measurement of VEGF. Sufficient quantities of all reagents are provided to yield 10 plates of 96 wells if the recommended assay procedure and recommended storage and handling of materials are followed as specified on this insert. The materials provided are **FOR RESEARCH USE ONLY**.

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

1. **Coating Antibody:** **Hu VEGF Coating Antibody (0.125mg/0.125mL)**
   - Part Number: AHG0114D
   - Lot Number: 728839
   - Form: Liquid, 1 vial, contains 0.1% sodium azide
   - Storage: Store at 2-8ºC for 1 month. For longer periods, aliquot and store at ≤ –20ºC.
   - Recommended Dilution: Dilute to 1.25 µg/mL with Coating Buffer B (Cat. # CB01100, or see Recommended Buffers). For example, to make 10 mL (enough to coat 1 plate), add 12.5 µL coating antibody to 9.988 mL Coating Buffer B.
2. **Detection Antibody:** **Hu VEGF Detection Antibody (0.025mg/0.125mL)**
   - Part Number: AHG9119D
   - Lot Number: 728836
   - Form: Liquid, 1 vial, contains 0.1% sodium azide
   - Storage: Store at 2-8ºC for 1 month. For longer periods, aliquot and store at ≤ –20ºC.
   - Recommended Dilution: Dilute to 0.025 µg/mL with Detection Antibody dilution buffer. (See formulation in Recommended Buffers and Solutions) For example, to make enough for 1 plate, add 1.25 µL detection antibody to 9.998 mL Buffer.
3. **Standard:** **Recombinant Hu VEGF**
   - Part Number: SD077 (inquire regarding additional vials)
   - Lot Number: 552961
   - Form: Lyophilized, 3 vials (single use)
   - Storage: Store at 2-8ºC.
   - Concentration of Reconstituted Standard: 10,000 pg/mL.
   - Reconstitution: Reconstitute in Assay Buffer (Cat. # DS98200 or see Recommended Buffers) according to instructions on vial label. Allow standard to rehydrate for approximately 10 minutes before dilutions. If the standard stock is not being used immediately, please aliquot into polypropylene tubes and freeze at –80ºC. *Do not store at room temperature or at 4ºC for any extended time or subject to more than one freeze-thaw cycle.*
   - Recommended Starting Standard Curve: Dilute standard stock to 1500 pg/mL (a 1:6.7 dilution) followed by six 1:2 serial dilutions using at least 300 µL of buffer. Mix thoroughly between dilutions. Avoid foaming. To an empty tube add 300 µL of buffer and label as zero standard.
4. **Streptavidin-HRP:** **0.025 mg/0.125 mL**
   - Part Number: SNN4004Y
   - Lot Number: 728913
   - Form: Liquid, 1 vial, contains animal serum and 50% glycerol in phosphate buffered saline with 0.05% thymol as a preservative.
   - Storage: Store concentrate at 2-8ºC for 1 month. For longer periods, aliquot and store at ≤ –20ºC. Diluted streptavidin-HRP should not be stored; discard remaining solution after use.
   - Recommended Dilution: Dilute to 0.05 µg/mL. For example, to make enough for 1 plate, add 2.5 µL of streptavidin-HRP to 9.9975 mL of Streptavidin-HRP solution (See formulation in Recommended Buffers and Solutions)



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Recommended Buffers and Solutions

1.	**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, q.s. to 1.0 L with distilled H_2O, pH to 9.4.
2.	**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; q.s. to 1.0 L with distilled H_2O, pH to 7.4.
3.	**Incubation Buffer**	***Incubation Buffer Formulation:*** 8.0 g NaCl, 0.2 g KCl, 3.4 g Na_2HPO_4, 0.6 g KH_2PO_4, 10.0 g bovine serum albumin (fraction V), 14.89 g EDTA; q.s. to 1.0 L with distilled H_2O
4.	**Detection Antibody Solution**	***Detection Antibody Solution Formulation:*** 8.77 g NaCl, 1.175 g Na_2HPO_4, 0.228 g NaH_2PO_4, 15mL Mouse serum, 10.0 g bovine serum albumin (fraction V); q.s. to 1.0 L with distilled H_2O, pH to 7.4.
5.	**Streptavidin-HRP Solution**	***Streptavidin-HRP Solution Formulation:*** 0.211g NaH_2PO_4, 1.388g Na_2HPO_4, 8.0 g NaCl, Thymol (**IMPORTANT!** Thymol must first be dissolved in absolute ethanol prior to addition. 5 mL thymol solution per liter of bulk is required for the batch. Dissolve 0.1 gram thymol per 1 mL ethanol. *Thymol is extremely toxic. Wear lab coat, gloves, face mask and safety goggles when handling),* 0.5 mL Tween 20, 20 g BSA; q.s. to 1.0L with distilled H_2O, pH to 7.5
6.	**Wash Buffer:**	***Wash Buffer (Cat. # WB01) from Invitrogen is recommended.*** *Alternate buffer choice listed below.* 9.0 g NaCl, 1 mL Tween 20; q.s. to 1.0 L with distilled H_2O, pH to 7.4.
7.	**Substrate Solution:**	***TMB (Cat. # SB01) from Invitrogen is recommended.*** *Alternate solution choice listed below.* Tetramethylbenzidine (TMB) and Hydrogen Peroxide.
8.	**Stop Solution:**	***Stop Solution (Cat.# SS03100) 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 300 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).
7. Pipette 50 uL of Incubation Buffer into all wells except for blank wells.
8. Pipette 100 µL of standards (in duplicate), samples and controls into designated wells. ***Cover plate and incubate for 2 hours at room temperature.***
9. Aspirate and wash 5 times using the method in step 3.
10. Pipette 100 µL of the working detection antibody into each well. For recommended dilutions, see "detection antibody" section. ***Cover plate and incubate for 1 hour at room temperature***
11. Aspirate and wash 4 times using the method in step 3.
12. Add 100 µL of the working streptavidin-HRP solution into each well. For recommended dilutions, see "streptavidin-HRP" section. ***Cover plate and incubate for 30 minutes at room temperature.***
13. Aspirate and wash 5 times using the method in step 3.
14. Add 100 µL of the TMB substrate to each well. ***Incubate plate <u>without</u> a plate cover for 30 minutes in the dark at room temperature.***
15. Add 100 µL of Stop Solution to each well.
16. 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.

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Additional Materials Required

- 96 well NUNC MaxiSorp microplates; NUNC Cat. # 434797 or Dynex Immulon 2 HB, Cat. #: 6506.
- Pipettes; plate covers or plate sealers 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.

Explanation of symbols

Symbol	Description	Symbol	Description
REF	Catalogue Number	LOT	Batch code
RUO	Research Use Only	IVD	*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer	EC REP	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.		

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