Mouse IL-6 Antibody Pair

10 Plate Format
Lot-specific Technical Data Sheet
Cat. No. CMC0063
Lot #*: 1755613

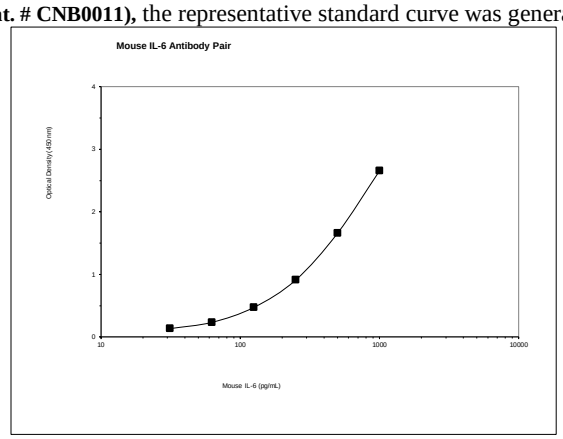
*Note: A letter at the end of the lot number signifies an additional packaging of this same lot.

Product Description and Materials Provided

The Antibody Pair for Mouse IL-6 contains components required to construct an enzyme-linked immunoassay for the specific and quantitative measurement of IL-6. 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.

1. **Coating Antibody:** **Anti-Mouse IL-6 (0.125 mg/0.125 mL)**
   - Part Number: AMC0864D
   - Lot Number: 1755615
   - 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:** **Anti-Mouse IL-6 Biotin (0.025 mg/0.125 mL)**
   - Part Number: AMC0969D
   - Lot Number: 1755616
   - 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 0.1 µg/mL with Assay Buffer (Cat. # DS98200, or see Recommended Buffers). For example, to make enough for 1 plate, add 3 µL detection antibody to 5.997 mL Assay Buffer.
3. **Standard:** **Recombinant Mouse IL-6**
   - Part Number: SD0302 (inquire regarding additional vials)
   - Lot Number: 1612210
   - Form: Lyophilized, 3 vials (single use)
   - Storage: Store at 2-8ºC.
   - Concentration of Reconstituted Standard: 10,000 pg/mL. Note: This standard has been calibrated against the WHO reference preparation 93/730 (NIBSC, Hertfordshire, UK, EN6 3QG0. One microgram equals 1260 arbitrary units.
   - Reconstitution: Reconstitute in Assay Buffer (Cat. # DS98200 or see Recommended Buffers) according to instructions on vial label. Allow standard to rehydrate for ~10 minutes before dilutions. If the standard stock is not used immediately, aliquot into polypropylene tubes and freeze at –80ºC. *Do not store at room temperature or 4ºC for an extended time or subject to more than one freeze-thaw cycle.*
   - Recommended Starting Standard Curve: Dilute standard stock to 1,000 pg/mL (a 1:10 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: 1791197
   - Form: Liquid, 2 vials, 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 0.1 µg/mL. For example, to make enough for 1 plate, add 5 µL of streptavidin-HRP to 9.995 mL of Assay Buffer (Cat. # DS98200 or see Recommended Buffers).

Following the recommended assay procedure using the **Invitrogen™ Antibody Pair Buffer Set (Cat. # CNB0011),** the representative standard curve was generated.



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) 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; q.s. to 1.0 L with distilled H_2O, pH to 7.4.
2. **Coating Buffer B:** ***Coating Buffer B (Cat. # CB01100) 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.
3. **Assay Buffer:** ***Assay Buffer (Cat. # DS98200) 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.
4. **Wash Buffer:** ***Wash Buffer (Cat. # WB01) 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.
5. **Substrate Solution:** ***TMB (Cat. # SB01) is recommended.*** *Alternate solution choice listed below.*
   Tetramethylbenzidine (TMB) and Hydrogen Peroxide.
6. **Stop Solution:** ***Stop Solution (Cat.# SS03100) is recommended.*** *Alternate solution choice listed below.*
   0.4 N H_2SO_4.

Assay Optimization

Antibody pairs 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).
7. Pipette 100 µL of standards (in duplicate), samples and controls into designated wells.
8. Immediately following step 7, add 50 µL of the working detection antibody into each well. For recommended dilutions, see "detection antibody" section. Gently tap the plate on the side 10 times to mix. ***Cover plate and incubate for 2 hours at room temperature.***
9. Aspirate and wash 5 times using the method in step 3.
10. 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.***
11. Aspirate and wash 5 times using the method in step 3.
12. 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.***
13. Add 100 µL of Stop Solution to each well.
14. 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.

Additional Materials Required

- 96 well NUNC MaxiSorp microplates; NUNC Cat. # 434797.
- 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	Symbol	Description
	Manufacturer		Catalog number		Batch code
	Use by		Temperature limitation		
	Consult instructions for use		Caution, consult accompanying documents		

For research use only. Not for use in diagnostic procedures.