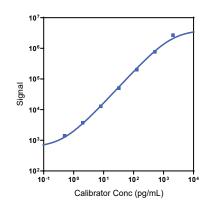
J-PLEX® NHP IL-4

| www.mesoscale.com [®] Ordering Information MSD Customer Service Phone: 1-240-314-2795 Fax: 1-301-990-2776 Email: CustomerService@ | Product Options | Catalog Number | Description | |
|---|-----------------|--|--|--|
| | Multiplex | K15068M, K25068M | U-PLEX Biomarker Group 1 (NHP) | |
| | | K156TWK-1/-2/-4 | U-PLEX NHP IL-4 Assay with SECTOR [™] plates | |
| | Singleplex | K156TWK-21/-22/-24 | U-PLEX NHP IL-4 Assay with QuickPlex Ultra [™] plates | |
| | | K256TWK-2/-4 | U-PLEX NHP IL-4 Assay with 384-well plates | |
| | Antibody Set | B26TW-2/-3 | U-PLEX NHP IL-4 Antibody Set | |
| | Assay Protocol | U-PLEX Product Inserts are available at <u>www.mesoscale.com</u> | | |

The MESO SCALE DISCOVERY[®] U-PLEX platform was designed to provide ultimate flexibility for detection of biomarkers in a wide variety of sample types. This datasheet provides the representative performance of the U-PLEX® NHP IL-4 Assay tested on U-PLEX 96-well SECTOR plates run as a multiplex. The data do not represent the product specifications. Under your experimental conditions, the assay may perform differently from the representative data. U-PLEX assays are offered in either singleplex or multiplex; both are available in 96- or 384-well plates. See a U-PLEX product insert for instrument compatibility.

Representative Calibration Curve and Sensitivity



| Assay | Median LLOD (pg/mL) | LLOD Range (pg/mL) | | |
|-------|------------------------|-----------------------|--|--|
| IL-4 | 0.06 | 0.03-0.11 | | |

The Calibrator curve was fitted with a 4-parameter logistic model with a 1/Y² weighting. The lower limit of detection (LLOD) is a calculated concentration corresponding to 2.5X the standard deviations above the background (zero Calibrator).

Precision

| | Control | Average Conc. (pg/mL) | Average Intra-run Conc. %CV | Inter-run Conc. %CV | |
|------|---------|-----------------------|-----------------------------|---------------------|--|
| | High | 772 | 4.1 | 9.5 | |
| IL-4 | Mid | 85 | 5.8 | 14.0 | |
| | Low | 8.7 | 5.9 | 16.4 | |

For Research Use Only. Not for use in diagnostic procedures.

Controls were made by spiking Calibrator into assay diluent at 3 levels within the quantitative range of the assay. Average intra-run concentration %CV is the average %CV of the control replicates within an individual run. Inter-run concentration %CV is the variability of controls across multiple runs.

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Spike Recovery

| | | Serum (N=5) | | Plasma (N=5) | | Cell Culture Media (N=5) | |
|----------------------|----------------|-----------------------|---------------------|-----------------------|---------------------|--------------------------|---------------------|
| | Spike Level | Average % Recovery | % Recovery Range | Average % Recovery | % Recovery Range | Average % Recovery | % Recovery Range |
| 0 | High | 90 | 76-100 | 83 | 76-91 | 99 | 88-107 |
| Cynomolgus Monkey | Mid | 103 | 98-104 | 90 | 87-95 | 97 | 88-109 |
| | Low | 108 | 100-112 | 91 | 87-95 | 102 | 97-109 |
| Dhaoua | High | 72 | 60-87 | 92 | 80-103 | | _ |
| Rhesus Monkey | Mid | 78 | 51-88 | 93 | 86-99 | _ | _ |
| | Low | 91 | 61-122 | 97 | 87-109 | — | _ |

Normal serum, EDTA plasma, and cell culture media were spiked with Calibrator at 3 levels. Undiluted samples were tested to determine the expected concentration of the analyte. Samples may benefit from additional dilution with assay diluent to reduce matrix effects. Dash (-) = not available

% Recovery = (measured concentration / expected concentration) x 100

Tested Samples

| | Sample Type | Serum (N=8) | Plasma (N=8) | Cell Culture Media (N=8) |
|----------------------|----------------|----------------|-----------------|-----------------------------|
| Cynomolgus Monkey | Median (pg/mL) | NA | NA | 53 |
| | Range (pg/mL) | NA | NA | ND-488 |
| | % Detected | 0 | 0 | 63 |
| Rhesus Monkey | Median (pg/mL) | NA | NA | 7.0 |
| | Range (pg/mL) | NA | NA | ND-211 |
| | % Detected | 0 | 0 | 63 |

Normal serum and plasma samples were diluted 2-fold prior to the assay. ND = not detectable (<LLOD); NA = not applicable due to 0% detected

Dilution Linearity

| | Serum (N=4) | | | Plasma (N=4) | | | Cell Culture Media (N=4) | | |
|----------------------|------------------|-----------------------|---------------------|------------------|-----------------------|---------------------|--------------------------|-----------------------|---------------------|
| | Fold Dilution | Average % Recovery | % Recovery Range | Fold Dilution | Average % Recovery | % Recovery Range | Fold Dilution | Average % Recovery | % Recovery Range |
| Cynomolgus Monkey | 2 | 105 | 88-120 | 2 | 102 | 88-121 | 2 | 113 | 103-123 |
| | 4 | 126 | 120-132 | 4 | 125 | 118-132 | 4 | 128 | 110-170 |
| | 8 | 132 | 119-142 | 8 | 128 | 120-134 | 8 | 138 | 115-181 |
| Rhesus Monkey | 2 | 123 | 111-147 | 2 | 112 | 101-122 | 2 | | _ |
| | 4 | 139 | 122-172 | 4 | 114 | 100-129 | 4 | | _ |
| wonkey | 8 | 146 | 127-192 | 8 | 123 | 113-137 | 8 | _ | |

Normal serum, EDTA plasma, and cell culture media were spiked with Calibrator and tested at different dilutions. Undiluted samples were tested to determine the expected concentration of the analyte. Samples may benefit from additional dilution with assay diluent to reduce matrix effects. dash = not available

% Recovery = (measured concentration / expected concentration) x 100





Specificity

To assess specificity, the IL-4 Antibody Set was tested individually against a larger panel of recombinant human analytes for nonspecific binding (CTACK, Eotaxin, Eotaxin-2, Eotaxin-3, ENA-78, FLT3L, Fractalkine, G-CSF, GM-CSF, GRO- α , I-309, IFN- α 2a, IFN- γ , IL-1 α , IL-1 β , IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-17A/F, IL-17B, IL-17C, IL-17D, IL-17F, IL-18, IL-22, IL-23, IP-10, I-TAC, MCP-1, MCP-2, MCP-3, MCP-4, M-CSF, MDC, MIF, MIP-1 α , MIP-1 β , MIP-3 α , MIP-3 β , MIP-5, SDF-1 α , TARC, TNF- α , TNF- β , TPO, TRAIL, VEGF-A, and YKL-40). Nonspecific binding was less than 0.5%.

% Nonspecificity = (nonspecific signal / specific signal) x 100

Diluent Compatibility

Diluents 57 and 3 are provided with this assay. MSD offers a range of assay and antibody diluents for separate purchase. Depending on your assay needs, other diluents may be tested.

Assay Components

Calibrator: IL-4 is included in Calibrator 1. The full-length recombinant protein is expressed in *E. coli*. **Antibodies:** The U-PLEX NHP IL-4 Assay uses a mouse monoclonal antibody for capture and a rat monoclonal antibody for detection. **Assay generation:** A

Note: This datasheet contains representative assay performance data. In custom multiplex formats, the assay may perform differently than the representative data shown.

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