U-PLEX®

NHP Eotaxin-3

Product Options	Catalog Number	Description
Multiplex	K15068M, K25068M	U-PLEX Biomarker Group 1 (NHP)
Singleplex	K156UEK-1/-2/-4	U-PLEX NHP Eotaxin-3 Assay with SECTOR™ plates
	K156UEK-21/-22/-24	U-PLEX NHP Eotaxin-3 Assay with QuickPlex® plates
	K256UEK-2/-4	U-PLEX NHP Eotaxin-3 Assay with 384-well plates
Antibody Set	B21UE-2/-3	U-PLEX Human Eotaxin-3 Antibody Set
Assay Protocol	U-PLEX Product Inserts are available at www.mesoscale.com	

The 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 Eotaxin-3 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
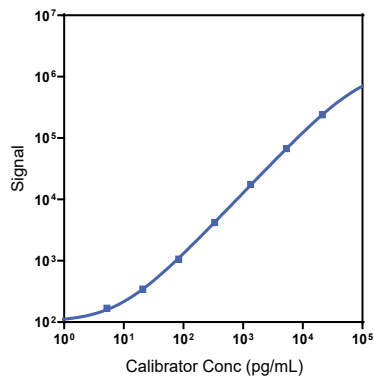
Assay	Median LLOD (pg/mL)	LLOD Range (pg/mL)
Eotaxin-3	3.26	1.2-4.52

The Calibrator curve was fitted with a 4-parameter logistic model with a $1/Y^2$ 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
Eotaxin-3	High	9,350	9.4	18.5
	Mid	914	7.2	18.1
	Low	95.3	7.5	14.9

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.

Spike Recovery

	Spike Level	Serum (N=5)		Plasma (N=5)		Cell Culture Media (N=5)	
		Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Cynomolgus Monkey	High	110	105-116	111.8	89-158	125	118-133
	Mid	105	100-111	107.7	61-160	128	101-142
	Low	103	97-111	105.9	54-170	128	95-145
Rhesus Monkey	High	137	116-150	138.7	131-146	125	118-133
	Mid	132	114-144	139.3	127-145	128	101-142
	Low	133	110-142	133	122-140	128	95-145

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.

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

Tested Samples

	Sample Type	Serum (N=10)	Plasma (N=10)	Spiked Serum (N=5)
Cynomolgus Monkey	Median (pg/mL)	8.09	NA	406
	Range (pg/mL)	ND-107	NA	387-457
	% Detected	20	0	100
Rhesus Monkey	Median (pg/mL)	ND	ND	293
	Range (pg/mL)	ND-9.60	ND-6.40	286-417
	% Detected	10	10	100

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=5)			Plasma (N=5)			Cell Culture Media (N=5)		
	Fold Dilution	Average % Recovery	% Recovery Range	Fold Dilution	Average % Recovery	% Recovery Range	Fold Dilution	Average % Recovery	% Recovery Range
Cynomolgus Monkey	2	91	86-95	2	99	89-109	2	92	86-99
	4	90	83-96	4	95	86-112	4	87	80-91
	8	88	81-93	8	95	84-116	8	81	74-87
Rhesus Monkey	2	111	109-114	2	88	81-94	2	92	86-99
	4	105	99-111	4	85	82-88	4	87	80-91
	8	110	105-118	8	79	77-83	8	81	74-87

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.

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

Specificity

To assess specificity, the Eotaxin-3 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-α2a, IFN-γ, IL‑1α, IL-1β, 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-1α, MIP‑1β, MIP-3α, MIP-3β, MIP-5, SDF-1α, 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: Eotaxin-3 is included in Calibrator 2. The full-length recombinant protein is expressed in *E. coli.*
Antibodies: The U-PLEX NHP Eotaxin-3 Assay uses a mouse monoclonal antibody for capture and a mouse monoclonal antibody for detection.
Assay generation: B

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

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