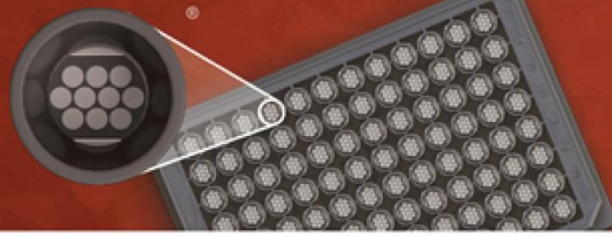
U-PLEX® NHP Eotaxin Assay



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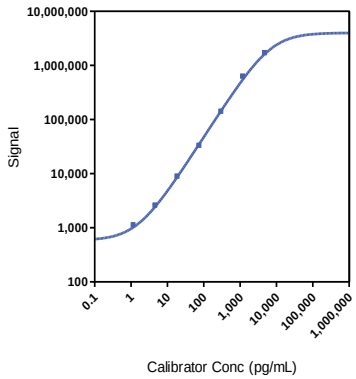
Product Options	Available as part of U-PLEX Biomarker Group 1 (NHP) multiplex combination: K15068L-1/-2/-4
	Individual assay: K156UDK-1/-2/-4; Antibody Set: B26UD-2/B26UD-3
	For more ordering options, please visit www.mesoscale.com
Instrument Compatibility	SECTOR® Imager 2400, SECTOR Imager 6000, MESO® SECTOR S 600, MESO QuickPlex® SQ 120
Sample Type	NHP (Cynomolgus monkey and Rhesus monkey) serum, EDTA plasma, and cell culture supernatants
Assay Protocol	Refer to the U-PLEX Biomarker Group 1 (NHP) product insert available at www.mesoscale.com/U-PLEX-documents

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 Assay tested on U-PLEX plates run as a multiplex. The data were generated during the development of the assay and do not represent the product specifications. Under your experimental conditions and with your specific multiplex, the assay may perform differently than the representative data shown. U-PLEX assays are available in multiplex format with other compatible assays. The same assay can also be used to detect a single analyte on MSD GOLD™ Small Spot Streptavidin plates.

Representative Calibration Curve and Sensitivity



Assay	Median LLOD (pg/mL)	LLOD Range (pg/mL)
Eotaxin	0.30	0.22-0.63

The calibration curves used to calculate analyte concentrations were established by fitting the signals from the Calibrators using a 4-parameter logistic (or sigmoidal dose-response) model with a $1/Y^2$ weighting. Analyte concentrations were determined from the electrochemiluminescence signals by back-fitting to the calibration curve. The lower limit of detection (LLOD) is a calculated concentration corresponding to the signal 2.5 standard deviations above the background (zero Calibrator).

Precision

	Control	Average Conc. (pg/mL)	Average Intra-run Conc. %CV	Inter-run Conc. %CV
Eotaxin	High	474	6.2	17.3
	Mid	47	8.1	18.8
	Low	4.4	11.2	22.2

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

		Serum		Plasma		Cell Culture Media	
	Spike Level	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Cynomolgus Monkey	High	110	90-140	78	71-82	110	104-114
	Mid	129	90-191	82	69-89	114	111-119
	Low	110	61-277	85	69-93	115	112-121
Rhesus Monkey	High	88	72-96	94	86-114	-	-
	Mid	101	89-111	100	90-108	-	-
	Low	102	99-106	96	85-108	-	-

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 require additional dilution with assay diluent to reduce matrix effects.
% Recovery = (measured concentration / expected concentration) x 100

Tested Samples

	Sample Type	Serum	Plasma	Stimulated PBMC Sample
Cynomolgus Monkey	Median (pg/mL)	411	140	NA
	Range (pg/mL)	280-11,028	119-221	NA
	% Detected	100	100	0
Rhesus Monkey	Median (pg/mL)	597	251	0
	Range (pg/mL)	169-1,453	79-516	ND-1.5
	% Detected	100	100	25

ND = non-detectable (< LLOD), NA = not applicable due to 0% detected

Normal serum and EDTA plasma samples were tested without dilution prior to the assay.

Dilution Linearity

	Serum			Plasma			Cell Culture Media		
	Fold Dilution	Average % Recovery	% Recovery Range	Fold Dilution	Average % Recovery	% Recovery Range	Fold Dilution	Average % Recovery	% Recovery Range
Cynomolgus Monkey	2	95	86-108	2	104	90-110	2	116	107-120
	4	108	98-116	4	123	115-136	4	127	120-143
	8	111	105-117	8	130	109-150	8	138	114-171
Rhesus Monkey	2	113	96-139	2	104	101-108	2	-	-
	4	124	102-162	4	101	97-109	4	-	-
	8	129	104-183	8	99	92-113	8	-	-

Normal serum, EDTA plasma, and cell culture media were spiked with recombinant Calibrator and tested at different dilutions. Undiluted samples were tested to determine the expected concentration of the analyte. Samples may require additional dilution with assay diluent to reduce matrix effects.
% Recovery = (measured concentration / expected concentration) x 100

Specificity

To assess specificity, the Eotaxin Antibody Set was tested individually against a larger panel of recombinant NHP analytes for nonspecific binding (CTACK, ENA-78, Eotaxin, Eotaxin-3, Fractalkine, G-CSF, GM-CSF, IFN-α2a, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-18, IP-10, I-TAC, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, MIP-3α, MIP-3β, SDF-1α, TARC, TNF-α, TNF-β, TPO, and VEGF-A). Nonspecific binding was less than 0.5%.

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

Diluent Compatibility

The data included in this document has been collected using Diluents 3 and 43. 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: NHP Eotaxin is included in Calibrator 2 blend. The full-length recombinant protein expressed in E.coli is used.
Antibodies: The U-PLEX NHP Eotaxin Assay uses mouse monoclonal antibody for capture and mouse 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.