Simultaneous Measurement of 67 Non-Human Primate Immunological, Metabolic, and Hormonal Biomarkers using Luminex® xMAP® Technology

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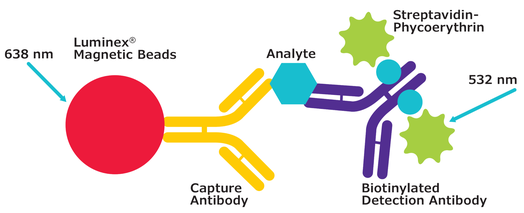
Introduction

Immune system, metabolic, and pituitary biomarker research in non-human primates (NHP) is essential for the study of immunological, metabolic, and hormonal diseases and regulation. The ability to test for multiple factors simultaneously in a single small sample is a valuable tool to researchers. To meet this need, we developed three NHP multiplex panels: MILLIPLEX® Non-Human Primate Metabolic Hormone (Cat. No. NHPMHMAG-46K), MILLIPLEX® Non-Human Primate Pituitary Panel 1 (Cat. No. NHPPT1MG-46K), and MILLIPLEX® Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A (Cat. No. PRCYTA-40K) for the simultaneous measurement of picogram levels of 12, 7, or 48 metabolic, pituitary, or immunological biomarkers, respectively, using Luminex® xMAP® technology. The MILLIPLEX® multiplex assays were tested for sample detection in healthy samples from 5 NHP species and with either LPS-challenged animals or fasted and post-feeding animal samples. Researchers will find the panels easy to use with straightforward protocols.

Methods

Microspheres. Magnetic microsphere beads were purchased from Luminex® Corp. Each set of beads is distinguished by different ratios of two internal dyes yielding a unique fluorescent signature to each bead set. Capture antibodies were covalently coupled to the carboxylate-modified magnetic microsphere beads.

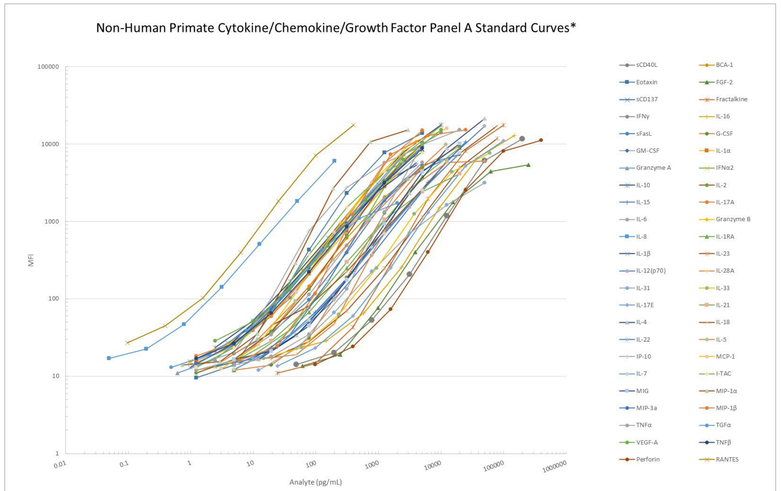


Typical MILLIPLEX® Immunoassay Protocol*:

- Prewet a 96-well plate with 200 µL Wash Buffer & decant
- Add 25 µL Standard or Sample (serum, plasma, cell culture, etc.)
- Add 25 µL Assay Buffer
- Add 25 µL Bead Mixture
- Shake overnight at 4 °C or 2 hr at RT
- Wash with Wash Buffer
- Add 25 µL Detection Antibody Mixture
- Shake 1 hour at RT
- Add 25 µL Streptavidin Phycoerythrin
- Shake 30 min at RT
- Wash with Wash Buffer
- Add 150 µL Sheath Fluid and read on Luminex® instrumentation
- Standard curve and sample value analyses performed with Belysa® Immunoassay Curve Fitting Software V1 (Cat. No. 40-122)

*There may be slight variations in volumes added between panels.

MILLIPLEX® 48-Plex NHP Cytokine/Chemokine/Growth Factor Panel A standard curves



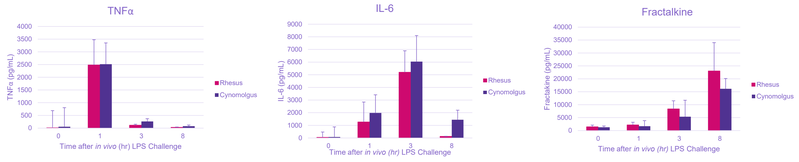
*Standard curves for all analytes were performed in MXPRSM-A serum matrix except RANTES, which was in L-AB assay buffer.

MILLIPLEX® NHP Cytokine/Chemokine/Growth Factor Panel A standard curve range and MinDC

Analyte	Standard Curve Range		MinDC (pg/mL)	Analyte	Standard Curve Range		MinDC (pg/mL)
	Min (pg/mL)	Max (pg/mL)			Min (pg/mL)	Max (pg/mL)	
sCD40L	49	200,000	38	IL-12(p70)	12	50,000	2.7
BCA-1	1.22	5,000	0.78	IL-28A	12	50,000	14.6
Eotaxin	1.22	5,000	1.35	IL-31	12	50,000	2.7
FGF-2	61	250,000	72	IL-33	15	60,000	7.9
sCD137	0.98	4,000	0.32	IL-17E	24	100,000	7.2
Fractalkine	24	100,000	10	IL-21	4.9	20,000	1.2
IFNγ	2.4	10,000	1.14	IL-4	12	50,000	3.0
IL-16	37	150,000	16	IL-18	20	80,000	4.3
sFasL	4.9	20,000	3.7	IL-22	2.4	10,000	1.1
G-CSF	4.9	20,000	3.9	IL-5	2.9	12,000	0.7
GM-CSF	1.22	5,000	0.47	IP-10	1.22	5,000	0.7
IL-1α	1.22	5,000	0.54	MCP-1	3.1	12,500	2.3
Granzyme A	0.61	2,500	0.27	IL-7	4.9	20,000	1.1
IFNα2	2.4	10,000	0.79	I-TAC	1.22	5,000	0.4
IL-10	2.4	10,000	0.49	MIG	4.9	20,000	2.4
IL-2	1.22	5,000	0.27	MIP-1α	0.73	3,000	1.0
IL-15	6.1	25,000	3.0	MIP-3a	1.22	5,000	0.4
IL-17A	1.22	5,000	0.33	MIP-1β	6.1	25,000	5.3
IL-6	1.22	5,000	0.48	TNFα	4.9	20,000	4.2
Granzyme B	0.98	4,000	0.22	RANTES*	0.10	400	0.1
IL-8	0.05	200	0.05	TGFα	0.49	2,000	0.1
IL-1RA	4.9	20,000	1.06	VEGF-A	2.4	10,000	0.5
IL-1β	0.98	4,000	0.25	TNFβ	1.22	5,000	0.3
IL-23	20	80,000	5.7	Perforin	98	400,000	62.5

*MinDC for all analytes were determined in MXPRSM-A serum matrix except RANTES was in L-AB assay buffer.

MILLIPLEX® NHP Cytokine/Chemokine/Growth Factor Panel A LPS *in vivo* challenged samples - Representative analyte time courses



Healthy Rhesus Monkeys (n=3) and Cynomolgus Monkeys (n=3), were dosed i.v. with 10 ug/kg LPS in 0.5 mL saline at time zero and serum samples were taken at 0, 1, 3 and 8hr (BioIVT). Samples were analyzed according to the protocol of the NHP Cytokine/Chemokine/Growth Factor Panel A. Representative analyte time course data (average and SEM) are shown.

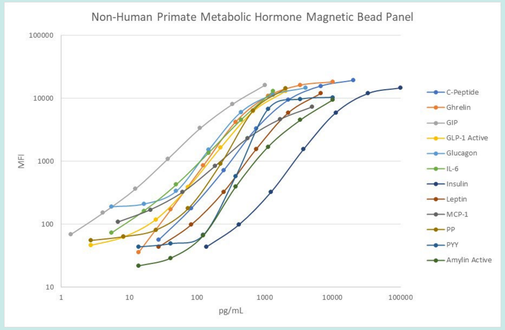
Detection of 5 NHP species using MILLIPLEX® NHP Cytokine/Chemokine/Growth Factor Panel A

	Analyte:	sCD40L	BCA-1	Eotaxin	FGF-2	sCD137	Fractalkine	IFNγ	IL-16	sFasL	G-CSF	GM-CSF	IL-1α	Granzyme A	IFNα2	IL-10	IL-2	IL-15	IL-17A	IL-6	Granzyme B	IL-8	IL-1RA	IL-1β	IL-23
Rhesus	Serum	+++	+++	+++	++	++	+++	++	++	++	++	++	++	++	++	++	+++	++	+++	++	+++	+++	++	+++	++
	Plasma	+++	+++	+++	-	+	+++	+	-	++	++	++	++	++	++	++	+++	-	-	+++	-	+++	++	+++	++
Cynomolgus	Serum	+++	+++	+++	++	++	++	++	+	++	++	++	++	++	++	++	+++	+	++	+++	+	+++	++	+++	++
	Plasma	+++	+++	+++	+	++	++	-	+	++	++	++	-	++	+	++	+++	++	++	++	-	+++	+++	++	++
Chimpanzee	Serum	+++	+++	+++	-	+++	+++	-	+++	-	+++	++	++	+++	++	+++	+	+++	+	-	+++	+	+++	+	+++	++
	Plasma	+++	+++	+++	-	++	++	-	++	-	++	+++	++	+	++	+++	+++	+	++	+++	+	+++	+++	+++	++
Baboon	Serum	+++	+++	+++	+	++	++	++	++	+++	++	+++	++	++	++	+++	+++	-	+++	+++	++	+++	+++	+++	++
	Plasma	+++	+++	+++	-	++	+++	++	++	+++	++	++	++	-	++	-	+++	+	++	++	-	+++	+++	+++	+
African Green	Serum	+++	+++	+++	-	++	+++	+	+	++	++	++	++	+	++	+	+++	+	+	+++	-	+++	+	+++	++
	Plasma	+++	+++	+++	-	++	+++	++	++	+++	++	++	++	-	+	-	+++	+	++	++	-	+++	+++	+++	+

	Analyte:	IL-12(p70)	IL-28A	IL-31	IL-33	IL-17E	IL-21	IL-4	IL-18	IL-22	IL-5	IP-10	MCP-1	IL-7	I-TAC	MIG	MIP-1α	MIP-3a	MIP-1β	TNFα	RANTES	TGFα	VEGF-A	TNFβ	Perforin
Rhesus	Serum	+	++	+	+	+++	+++	++	++	++	++	+++	+++	++	+++	++	++	++	+++	++	+++	+++	++	+++	+++
	Plasma	-	+++	-	-	+++	+++	+	+	+++	+	+++	+++	-	+++	-	+++	+	+++	+	+++	-	+	+++	+++
Cynomolgus	Serum	+	++	+	-	+++	+++	+	-	++	++	+++	+++	++	+++	+	+++	++	+++	++	+++	+++	+	++	+++
	Plasma	-	++	++	++	+++	+++	+	+	++	++	+++	+++	+	+++	+	+++	++	+++	++	+++	+++	+	+++	+++
Chimpanzee	Serum	-	++	+	-	+++	+++	+	++	++	-	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+	++	+++
	Plasma	+	+++	+	-	+++	+++	+	+++	++	-	+++	+++	+	+++	+++	+++	++	+++	++	+++	+++	+++	+	+++	+++
Baboon	Serum	+	++	+++	++	+++	+++	+++	-	+++	+	+++	+++	-	+++	+++	+++	+++	+++	++	+++	+++	+++	++	+++	+++
	Plasma	-	+++	++	++	+++	+++	++	-	++	++	+++	+++	+	+++	+++	+++	++	+++	+	+++	+++	++	+++	+++	+++
African Green	Serum	-	++	+	-	+++	+++	+	-	++	+	+++	+++	-	+++	+	++	++	+++	++	+++	+++	++	++	+++	+++
	Plasma	-	++	+	-	+++	++	-	-	++	++	+++	+++	-	+++	-	-	+++	+++	++	+++	+++	+	+++	+++	+++

Healthy animal Rhesus (10 serum/4 plasma), Cynomolgus (8 serum/4 plasma), Chimpanzee (5 serum/4 plasma), Baboon (5 serum/4 plasma), and African Green (5 serum/4 plasma) samples (BioIVT), were tested following the protocol of the MILLIPLEX® Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A. +++ indicates all samples were detected, ++ more than one but not all samples detected, + at least one sample were detected, - no samples were detected.

MILLIPLEX® 12-Plex NHP Metabolic Panel

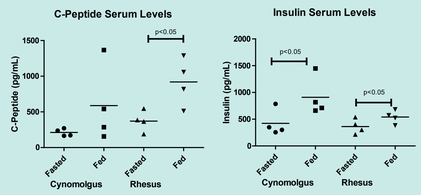


Analyte	Standard Curve Range	Sensitivity (MinDD)
C-Peptide	27-20,000 pg/mL	2.7
Ghrelin	14-10,000 pg/mL	1.8
GIP	1.4-1,000 pg/mL	5.3
GLP-1 Active	2.7-2,000 pg/mL	1.8
Glucagon	5.5-4,000 pg/mL	7.1
IL-6	5.5-4,000 pg/mL	3.5
Insulin	137-100,000 pg/mL	4.4
Leptin	27-20,000 pg/mL	1.8
MCP-1	7-5,000 pg/mL	0.9
PP	2.7-2,000 pg/mL	2.7
PYY	14-10,000 pg/mL	5.3
Amylin Active	14-10,000 pg/mL	4.4

Analyte:	C-Peptide	Ghrelin*	GIP	GLP-1 Active*	Glucagon*	IL-6	Insulin	Leptin	MCP-1	PP	PYY	Amylin Active*
African Green	+	-	++	++	++	-	+	+++	+++	+++	+	-
Baboon	+++	-	+++	++	++	-	++	++	+++	+++	+	+
Chimpanzee	+	-	++	+++	++	-	+	+++	+++	+++	-	-
Cynomolgus	+++	+	+++	+++	++	-	+++	++	+++	+++	+	+
Rhesus	+++	-	+++	+++	++	-	++	++	+++	+++	+	-

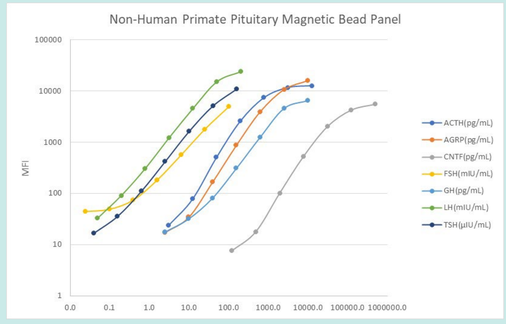
*sample did not contain inhibitors

Healthy animal serum and plasma (BioIVT), were tested following the protocol of the MILLIPLEX® Non-Human Primate Metabolic Hormone Panel.



Unmatched serum samples were taken from healthy Cynomolgus Monkeys (n=4) and Rhesus Monkeys (n=4) (BioIVT) after fasting overnight and after feeding, and then tested following the protocol of the MILLIPLEX® Non-Human Primate Metabolic Hormone Panel.

MILLIPLEX® 7-Plex NHP Pituitary Panel 1



Analyte	Standard Curve Range	Sensitivity (MinDD)
ACTH	3.1-12,500 pg/mL	<3.1
AGRP	2.4-10,000 pg/mL	<2.4
CNTF	122-500,000 pg/mL	2
FSH	0.02-100 mIU/mL	2.65
GH	2.4-10,000 pg/mL	7.07
LH	0.05-200 mIU/mL	0.88
TSH	0.04-160 µIU/mL	1.77

Analyte:	ACTH	AGRP	CNTF	FSH	GH	LH	TSH
African Green	+	++	++	+++	+++	-	+++
Baboon	-	++	+++	+++	+++	-	+++
Chimpanzee	-	++	++	+++	+++	+++	-
Cynomolgus	-	+	+++	+++	+++	-	+++
Rhesus	-	+	++	++	+++	+++	+++

Healthy animal serum and plasma (BioIVT) were tested following the protocol of the MILLIPLEX® Non-Human Primate Pituitary Panel 1.

Summary

Three MILLIPLEX® NHP Panels: Non-Human Primate Metabolic Hormone (Cat. No. NHPMHMAG-45K), Non-Human Primate Pituitary Panel 1 (Cat. No. NHPPT1MG-46K), and Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A (Cat. No. PRCYTA-40K) have been developed to meet the needs of researchers for the study of non-human primate metabolic, pituitary, and immunological biomarkers. Representative data shown here exemplifies the value of these kits for the study of relevant biomarkers in serum and plasma in five species of non-human primates. The MILLIPLEX® Non-Human Primate Panels are powerful tools for researchers for the profiling of non-human primate biomarkers.

Discover more at SigmaAldrich.com/milliplex-primate

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