Abstract
Cytokines are soluble proteins that act as extracellular signaling molecules and mediate communications between cells. They regulate the immune system, inflammation, trauma, and cancer, as well as cell growth, migration, development and differentiation. Infections with HIV results in dysregulation of the cytokine profile and certain cytokines can modulate HIV expression and replication. Due to the immunological and physiological similarities between humans and non-human primates (NHPs), understanding the disease progression and immune response in NHP species help predict the clinical response in AIDS patients. Therefore, accurate measurement of cytokines in primate models is important to advance the development of vaccines and anti-retroviral drugs for AIDS prevention and treatment.

We have developed several NHP multiplex panels for the simultaneous quantification of a variety of NHP biomarkers. Our panels include interleukins (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p40, IL-17A, IL-21, IL-23), IFN-γ, TNF-α, chemokines (RANTES, MCP-1, MIP-1α, MIP-1β, IP-10, IL-8, MIG and I-TAC), growth factors (EGF, G-CSF, GM-CSF, PDGF-AA, PDGF-BB, SCF, HGF, M-CSF, TGF-α, VEGF, EPO, FGF-basic, Angiopoietin-2), and adipokines (Adiponectin, Adipin, Resistin, Leptin). Our multiplex assays are bead-based sandwich immunoassays using fluorescence-encoded beads that can be separated by a variety of flow cytometers. In each panel, up to thirteen NHP biomarkers can be quantitated simultaneously. All panels have been validated for use in cell culture supernatant, serum, plasma and urine samples for both rhesus and cynomolgus monkey species. These panels provide higher sensitivities and broader dynamic ranges than traditional ELISA methods, offering useful tools for researchers dedicated to HIV research in NHPs.

Assay Protocol
25 µL Assay Buffer or serum matrix
25 µL Standard or samples (diluted or neat)
25 µL beads
Incubate with shaking for 2h at RT or O/N at 4°C
Wash twice
25 µL Detection antibody
Incubate with shaking for 30 min at RT
25 µL Streptavidin-Phycocerythrin
Incubate with shaking for 30 min at RT
Wash twice
Read on a Standard flow cytometer
Data analyzed with free software

Principle of the Assay
Bead-Based Sandwich Immunoassay:

Results, Biological Samples (pg/mL)
Cynomolgus (Cyn.) and Rhesus (Rhes.) monkey PBMCs were stimulated and cultured under various conditions as listed, and the supernatants were removed and assayed after 3 days. NHP biomarkers in PBMC culture supernatant were determined using LEGENDplex® assays.

Conclusions
1. We have developed new bead-based multiplex assay panels for simultaneous quantification of soluble NHP biomarkers, including cytokines, chemokines, growth factors and adipokines.
2. These assays are analytically robust, with high sensitivities, wide dynamic ranges, great accuracy and reproducibility. The assay results are read with flow cytometers commonly available for cell analysis. Simple, fast, and accurate data analysis is enabled with complimentary software available from BioLegend.
3. The utility of the custom multiplex assays has been further validated by using relevant biological samples.