LakePharma
The Biologics Company

Assay and Workflow Development Services for Discovery and GxP Processes

LakePharma Assay Development Team and Facility

- Facility located in Hopkinton, MA, conveniently close to Boston/Cambridge areas
- Team composition
  - 22+members with >70% holding PhDs or MS
  - Team led by Douglas Demian, Pfizer Alumnus, Early Discovery Assay Development and Screening at DTC/RTC in Cambridge, MA 2000-2008.
- Team experienced a wide variety of techniques and technologies including:
  - Development of cell-based and enzymatic assays
  - qPCR
  - Primary Cell Culture/iPSC
  - Radioactive endpoints and optimization of complex assay workflows

LakePharma Hopkinton
79,000 sq.ft. cGMP/R&D site

Workflow Support Modalities

LakePharma can initiate customer projects from a variety of starting points

- **Literature references**
- **Drug target and desired experimental readout**
- **Modification of existing assay for different target**
- **Assay transfer and optimization (existing working assay)**

- Higher level of complexity & ambiguity
- Longer project length, higher overall cost

- Lower level of complexity, defined endpoint
- Shorter project length, lower overall cost

Workflow Support Modalities

LakePharma can collaborate with clients in various ways

- **Full Time Equivalent (FTE) Workflows**
  - One or more LakePharma staff dedicated to client workflow
  - Typically longer term relationships, full development/discovery campaigns with multiple projects
  - Ideal for mix of development and routine assay execution
  - Ultimate flexibility for client workflow with true scientific partnership from dedicated LakePharma team resource
- **Fee-for-Service Workflow**
  - Typically the entry point with clients new to assay workflows at LakePharma
  - Small projects, commercial kit-based sample endpoint testing to larger development projects
  - Each workflow custom quoted to meet project goals
- **Blanket PO and/or À la carte Quote**
  - Ideal for optimized workflows following assay development
  - Also applicable for assay workflows that require “as-needed” testing at varying times throughout the year.

- Increased workflow flexibility
- Increased level of scientific partnership

- Iterative testing workflows with defined endpoints

We will build capacity and add instrumentation to suit your workflow.

LakePharma
The Biologics Company

Assay Types Supported

Cell-Based Assays

- Platform assays: ADCC/CDC, cytotoxicity, T cell functional (stim, reg, CMV recall), Ab neutralization assays, cytotoxicity
- PBMC-based workflows (commercial vendors and on-site donor program)
- Custom development functional assays
- Reporter gene/immune checkpoint assays
- High content, image-based assays
- Whole-cell radiometric assays
- HTS/secondary assay support (multimode reader or HT flow cytometry)
- Custom bioassay cell line generation, RCB generation (cGxP available)

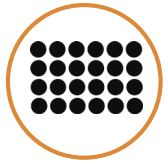
Biochemical Assays

- Enzyme activity/kinetics
- Binding affinity determinations (ELISA, TMB, BLI)
- Immunoassays (MSD, TMB, BLI)
- Membrane prep/biochemical radiometric assays
- HTS/secondary assay support
- Custom biochemical assay development
- Bulk tissue processing (human, mouse/rat, Cyno, others)

Assay Types Supported (cont'd)

Genetic Technology Suite

- qPCR expression profiling (TaqMan or SYBR Green technology)
- Custom reagent design and validation (qPCR/ddPCR/Nano)
- Sanger sequencing
- ddPCR workflows (BioRad QX200 AutoDG System)
- NGS/RNAseq workflows (3rd party collaborator)
- NanoString FLEX DX System (cGxP)



Multi-Analyte Array Workflows:

- MSD ECL ELISAs
- NanoString FLEX DX System (cGxP)

GxP Bioassay Development Workflows

- Bridging of R&D assays to GxP environment
- Full protocol development and data package delivery

Radiometric Workflows

- ^{3}H, ^{14}C, ^{32}P, ^{33}P, ^{35}S, ^{51}Cr, ^{125}I isotopes
- Plate, vial, and electrophoretic-based biochemical, cell-based, and analytical readouts

Select Instruments & Systems

LakePharma utilizes a wide range of industry-leading systems to support your assay development needs.

Microplate Readers

- Meso Scale Discovery Sector S 600
- BMG PHERAstar, BioTek Cytation 5, SpectraMax M5 Multimode Readers
- PerkinElmer Trilux Microplate Scintillation Reader

Cell Imaging, Analysis and Sorting

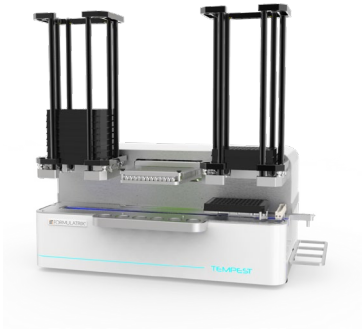
- BD Accuri C6+ Cytometer (4-color)
- Intellicyt iQue Screener Plus (4-color)
- Partnership with UMass Medical FACS core facility
- BioTek Cytation 5 (HCS w/ CO_2 and Injectors)
- Zeiss Axio Observer 1 and 7 Fluorescent Microscopes

Genetic Analysis Technology

- QuantStudio 6 qPCR
- NanoString FLEX DX System (cGxP)
- BioRad QX200 AutoDG ddPCR (cGxP)
- ABI 7500 DX qPCR and 3500 DX Genetic Analyzer (cGxP)

Liquid Handling/Washers

- Formulatrix Tempest Automated Dispenser Platform
- Integra BioSciences ViaFlo96/384 pipettors
- Apricot i-Pipette 384 well pipettor
- Biotek Elx 405 plate washer w/stacker
- Hewlett Packard d300e Digital Dispenser
- Labcyte Echo 550 (3rd Party Collaboration)



Gel Imagers / BLI / Other Imagers

- Azure c400 Imager, RGB (cGMP)
- Octet HTX 384
- Typhoon Trio RGB Phosphorimager
- PerkinElmer Tricarb 4910 Scintillation Counter

Analytical Instruments

- Agilent 1100 HPLC
- ProteinSimple Maurice cIEF/ceSDS (cGxP)

LakePharma
The Biologics Company

Key Summary of LakePharma Services

- Broad experience in assay development and screening workflows
- Large portfolio of technologies, formats, and readouts
- Demonstrated record of success in recurrent assay support and developmental workflows
- Flexibility in capacity, assay design, and execution to meet your data requirements

Ultimately, your assay, your way.

Contact Us Today

888-406-5658 (Toll free)

Inquiries@LakePharma.com

www.lakepharma.com

LakePharma
The Biologics Company

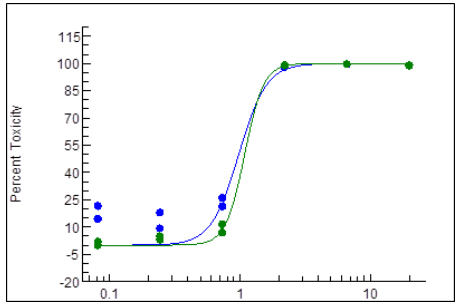
Backup Slides

Small Molecule Discovery Case Study

Development and Screening of an HepG2 Cytotoxicity Assay in Two Conditions—Glucose and Galactose Growth Conditions (Crabtree Effect*)

- **Purpose:** First pass cytotoxicity assay for newly synthesized SAR compounds in a variety of small molecule target programs
- **Design:** 384-well luminescence-based assay with HepG2 cells maintained in standard glucose media and galactose media to identify compounds that show “crabtree” effect (reduced sensitivity to cytotoxic compounds in glucose media)
- **Optimization Work:** Transitioned original 96-well assay to 384-well 6-point, 4-fold titrations to maximize compound density while maintaining overall signal/background and screening window
- **Outcome:** Supporting screening efforts for 2+ years at approximately 20 compounds/week in the two culture conditions

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	7.11E+02	1.81E+03	9.09E+03	9.86E+05	1.09E+06	1.09E+06	2.05E+04	7.55E+05	1.10E+06	1.13E+06	1.19E+06	1.15E+06	1.90E+04	7.08E+05	1.23E+06	1.36E+06	1.23E+06	1.25E+06	1.45E+04	1.19E+04	1.11E+06	1.15E+06	1.27E+06	1.31E+06
B	1.09E+03	2.27E+03	1.19E+04	1.14E+06	1.13E+06	1.26E+06	3.40E+04	1.02E+06	1.26E+06	1.20E+06	1.38E+06	1.18E+06	2.24E+04	7.45E+05	1.15E+06	1.31E+06	1.33E+06	1.29E+06	1.66E+04	1.35E+04	1.13E+06	1.13E+06	1.31E+06	1.28E+06
C	1.28E+03	3.03E+03	1.28E+04	1.12E+06	1.27E+06	1.34E+06	1.32E+06	1.21E+06	1.37E+06	1.24E+06	1.38E+06	1.39E+06	2.08E+04	1.90E+04	8.28E+05	1.27E+06	1.22E+06	1.23E+06	1.74E+04	7.66E+03	1.51E+04	1.65E+04	1.70E+04	1.51E+04
D	1.55E+03	4.49E+03	1.85E+04	1.11E+06	1.22E+06	1.35E+06	1.19E+06	1.37E+06	1.19E+06	1.20E+06	1.37E+06	1.14E+06	1.87E+04	1.28E+04	5.10E+05	1.24E+06	1.18E+06	1.18E+06	1.68E+04	9.45E+03	1.50E+04	1.74E+04	1.71E+04	1.52E+04
E	2.21E+03	1.01E+04	9.01E+05	1.24E+06	1.32E+06	1.36E+06	1.15E+06	1.28E+06	1.16E+06	1.20E+06	1.35E+06	1.20E+06	1.89E+04	9.58E+03	3.45E+04	1.10E+06	1.34E+06	1.16E+06	1.76E+04	1.51E+04	1.19E+06	1.29E+06	1.40E+06	1.41E+06
F	2.36E+03	1.17E+04	9.46E+05	1.09E+06	1.22E+06	1.20E+06	1.04E+06	1.08E+06	1.34E+06	1.31E+06	1.23E+06	1.12E+06	1.86E+04	9.70E+03	2.35E+04	1.17E+06	1.22E+06	1.27E+06	1.87E+04	1.86E+04	1.25E+06	1.36E+06	1.52E+06	1.55E+06
G	2.82E+03	1.16E+04	9.42E+05	9.49E+05	1.26E+06	1.18E+06	1.20E+06	1.10E+06	1.34E+06	1.14E+06	1.36E+06	1.30E+06	2.06E+04	1.51E+04	9.49E+05	1.16E+06	1.21E+06	1.16E+06	1.96E+04	1.13E+04	2.83E+04	1.40E+06	1.46E+06	1.44E+06
H	3.04E+03	1.24E+04	9.74E+05	1.08E+06	1.15E+06	1.20E+06	1.22E+06	1.31E+06	1.31E+06	1.23E+06	1.19E+06	1.33E+06	2.59E+04	1.79E+04	9.70E+05	1.13E+06	1.18E+06	1.18E+06	2.96E+04	2.31E+04	2.94E+04	1.45E+06	1.47E+06	1.47E+06
I	3.23E+03	1.24E+04	1.11E+06	1.29E+06	1.31E+06	1.21E+06	2.93E+04	2.73E+04	9.90E+05	1.15E+06	1.15E+06	1.22E+06	9.40E+05	2.98E+04	1.03E+06	1.14E+06	1.27E+06	1.35E+06	1.49E+06	1.50E+06	1.51E+06	6.66E+04	2.19E+04	1.81E+04
J	3.57E+03	1.29E+04	1.10E+06	1.30E+06	1.38E+06	1.36E+06	2.12E+04	1.78E+04	1.09E+06	1.17E+06	1.18E+06	1.23E+06	9.57E+05	1.02E+06	1.04E+06	1.22E+06	1.39E+06	1.41E+06	1.47E+06	1.47E+06	1.56E+06	5.94E+04	2.00E+04	1.88E+04
K	3.51E+03	1.32E+04	1.03E+06	1.31E+06	1.28E+06	1.35E+06	2.03E+04	1.35E+04	5.09E+05	1.14E+06	1.28E+06	1.26E+06	2.84E+04	2.67E+04	1.17E+06	1.21E+06	1.35E+06	1.48E+06	3.22E+05	6.76E+05	3.21E+05	8.37E+05	1.40E+06	1.59E+06
L	3.84E+03	1.43E+04	1.07E+06	1.44E+06	1.34E+06	1.39E+06	2.07E+04	1.22E+04	2.56E+05	1.14E+06	1.32E+06	1.38E+06	2.21E+04	2.01E+04	1.17E+06	1.29E+06	1.38E+06	1.47E+06	2.74E+05	7.14E+05	6.08E+05	9.89E+05	1.49E+06	1.63E+06
M	4.21E+03	1.54E+04	1.33E+06	1.41E+06	1.39E+06	1.51E+06	2.19E+04	1.68E+04	1.12E+06	1.27E+06	1.46E+06	1.46E+06	2.29E+04	2.09E+04	1.26E+06	1.48E+06	1.51E+06	1.54E+06	1.56E+06	1.54E+06	1.59E+06	1.62E+06	1.55E+06	1.66E+06
N	1.10E+04	2.38E+04	1.33E+06	1.40E+06	1.41E+06	1.43E+06	2.21E+04	2.20E+04	1.17E+06	1.19E+06	1.43E+06	1.48E+06	2.16E+04	1.92E+04	1.23E+06	1.51E+06	1.46E+06	1.55E+06	1.63E+06	1.60E+06	1.60E+06	1.63E+06	1.60E+06	1.58E+06
O	9.74E+05	1.25E+06	1.15E+06	1.32E+06	1.34E+06	1.44E+06	2.54E+04	9.11E+05	1.03E+06	1.15E+06	1.42E+06	1.45E+06	2.04E+04	1.19E+04	3.86E+05	1.35E+06	1.39E+06	1.47E+06	1.52E+06	1.49E+06	1.58E+06	1.53E+06	1.56E+06	1.55E+06
P	1.01E+06	1.22E+06	1.27E+06	1.32E+06	1.41E+06	1.46E+06	1.78E+04	3.43E+04	1.20E+06	1.12E+06	1.46E+06	1.49E+06	1.69E+04	9.21E+03	4.12E+05	1.36E+06	1.37E+06	1.49E+06	1.51E+06	1.56E+06	1.57E+06	1.56E+06	1.45E+06	1.59E+06



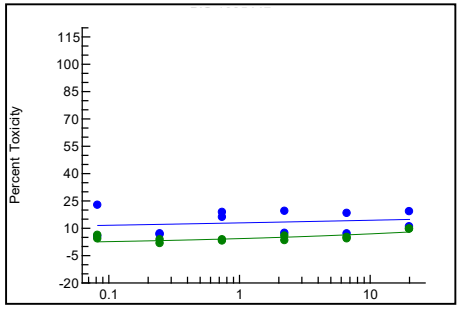


Plate z-factor
0.878

Average	1.47E+06	2.88E+04
Std.Dev.	4.19E+04	1.69E+04

TOXICOLOGICAL SCIENCES 97(2), 539–547 (2007)

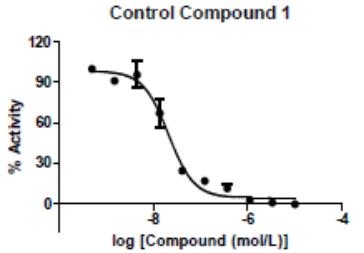
LakePharma
The Biologics Company

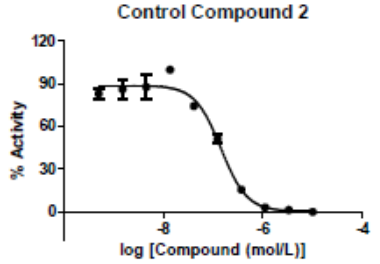
Small Molecule Discovery Case Study

Development and Recurrent Screening in a Cell-Based Phospholipase Assay (MSD)

- **Purpose:** Support of secondary screening for SAR activities on a phospholipase target program
- **Design:** Ramos cells stimulated with IgM +/- compound treatment followed by lysis and detection of phosphorylated target by sandwich ELISA on MSD platform
- **Optimization Work:** Full development work on cell handling, passage densities and passage limits to ensure functional response to experimental stimulus and optimal signal-to-noise ratio of assay
- **Outcome:** Supported 30+ compounds/week in 10-Point, 3-Fold Duplicate Titrations

STIMULATED											NO STIM
1	2	3	4	5	6	7	8	9	10	11	12
3455	13596	11395	21029	27606	36657	41885	41491	41268	38196	34679	2465
5013	15213	15002	29097	29814	40484	40640	36896	35087	37015	32688	3541
4616	6289	6129	10054	15114	28756	31724	36633	42823	37580	34495	3613
4259	7236	7541	13709	14553	22485	34759	36261	40615	39799	34162	3210
1690	2459	2640	5102	8412	14432	21499	31137	36691	34100	36179	4343
1717	2667	2722	5923	9370	15296	25774	34372	36438	35461	36513	4631
7806	13554	15720	31768	41797	45324	38960	43443	39514	39928	37109	3871
8611	13222	12936	27253	38512	38692	34962	41771	37617	38386	36015	4078
									ave	35230	3719
					Z'-score	0.795139			sd	1471	681
									%CV	4.17	18.32



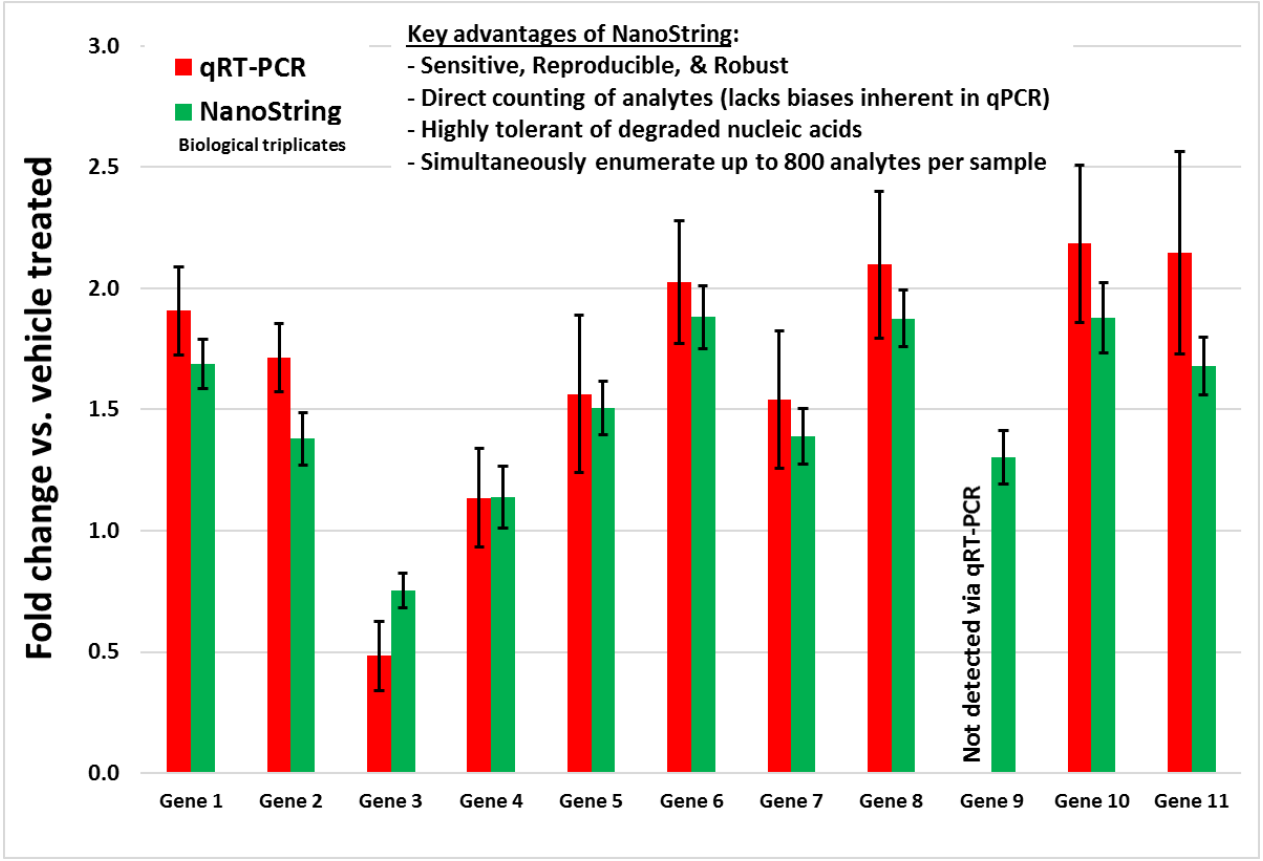


LakePharma
The Biologics Company

Assay Development Case Study

Comparison of NanoString vs qPCR (TaqMan-Based)

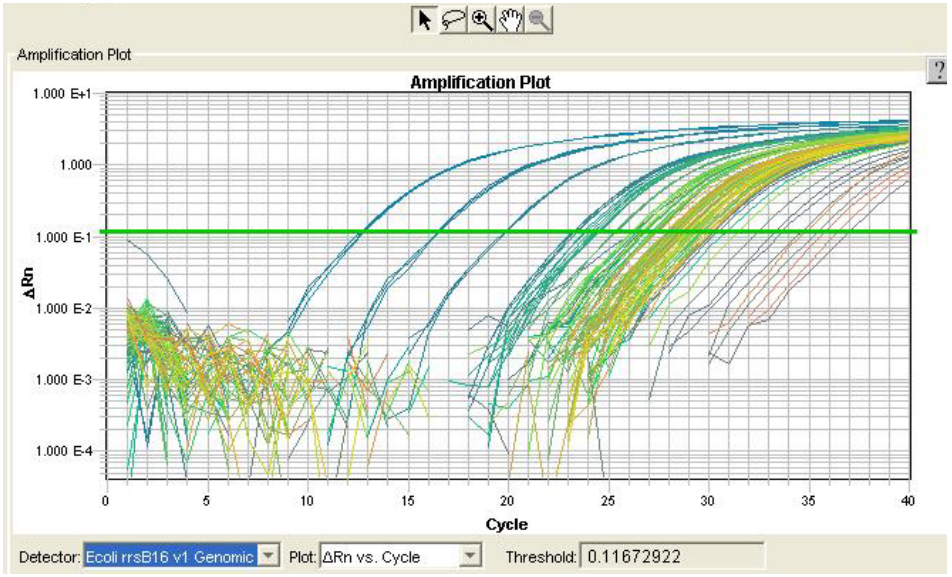
- **Purpose:** Evaluation of NanoString analysis against traditional qPCR methodology
- **Design:** Cell-based workflow comparing inventoried TaqMan Assays versus CodeSets from NanoString for same analytes
- **Workflow:** Minimal optimization required for NanoString execution. Good quality DNA/RNA prep.
- **Outcome:** Currently support 20 to 800 analyte/sample analyses in a variety of cell-based, tissue-based experimental workflows

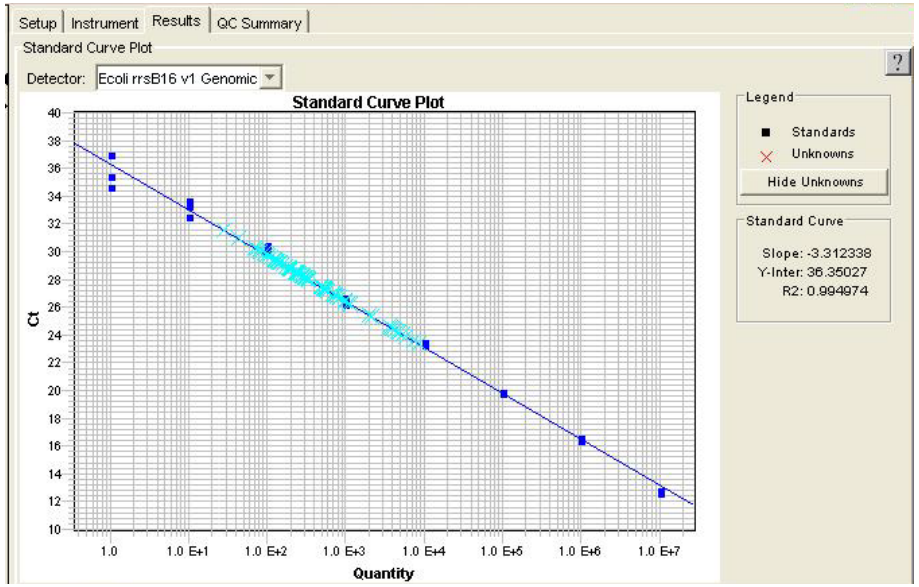


Process Development Case Study

Development of qPCR-Based Method for Determination of Residual DNA in Process Fluids

- **Purpose:** Client's process for generating RNA therapeutic was contaminated with residual plasmid DNA and genomic DNA. Accurate and specific methodology needed for assessing levels of contamination for process development efforts with the ultimate goal of transferring protocol to Client's QA Group
- **Design:** Absolute quantitation with plasmid-based standard curves. Custom designed multiple probe and primer sets for client's sequence of interest.
- **Optimization Work:** Validated probe sets and performed assessment of sample matrix influence on data output as well as spike/recovery tests with standard plasmids
- **Outcome:** Currently in recurrent support of assay for client and also have developed more analogous assays for additional analytes



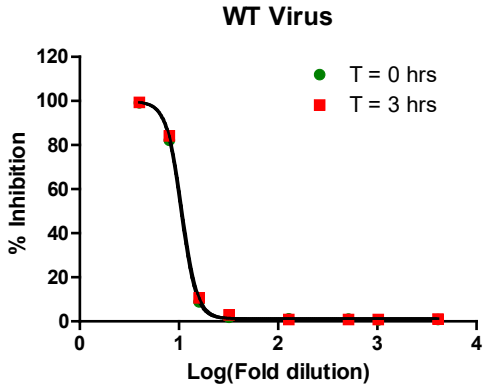


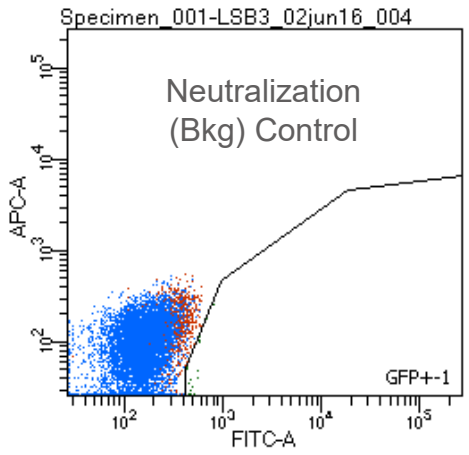
LakePharma
The Biologics Company

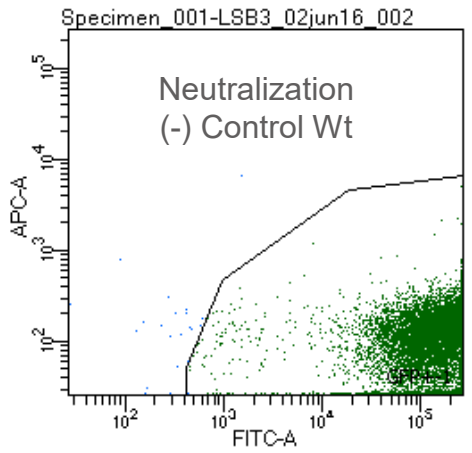
Antibody Discovery Case Study

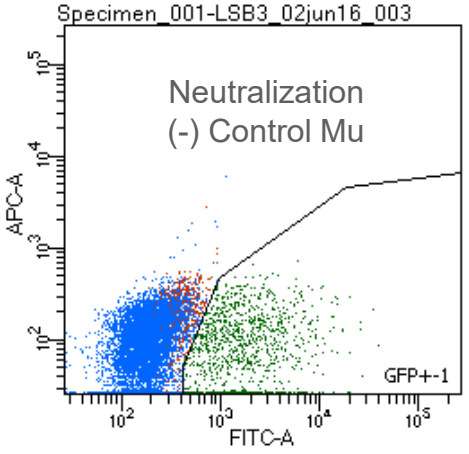
Antibody Neutralization Assay in HEK293 Cells

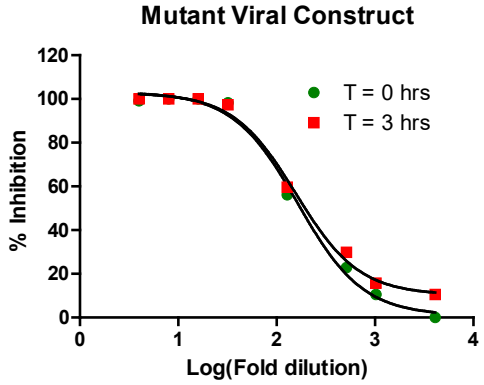
- **Purpose:** Recurrent flow cytometry screening of non-human primate serum samples for neutralizing antibodies to a particular set of viruses in support of Client's Rare Disease Unit
- **Design:** HEK293 cells are transduced with MOI of 10^4 virus particles either neat or pre-incubated with heat-inactivated test serum samples in titration for 48 hours to determine "IC_{50}" for neutralization of transduction. Readout by flow cytometry to detect virally expressed GFP. Wt virus capable of 100% transduction @ 10^4 MOI; Mutant-only capable of ~7-14% transduction @ 10^4 MOI
- **Optimization Work:** Confirmation of functionality of Wt and Mutant viruses and establish consistency with existing literature data for transduction levels. Scaling of workflow for maximum sample throughput
- **Outcome:** Currently in recurrent support modality; batches of 50-150 samples. Additional viral variants optimized and in testing











LakePharma
The Biologics Company