R&D systems™
by biotechne

Meet Leo

The Simple Way to Maximize Throughput for Reproducible Protein Quantitation with Size and Identity Confirmation

Reimagine Protein Analysis with Leo

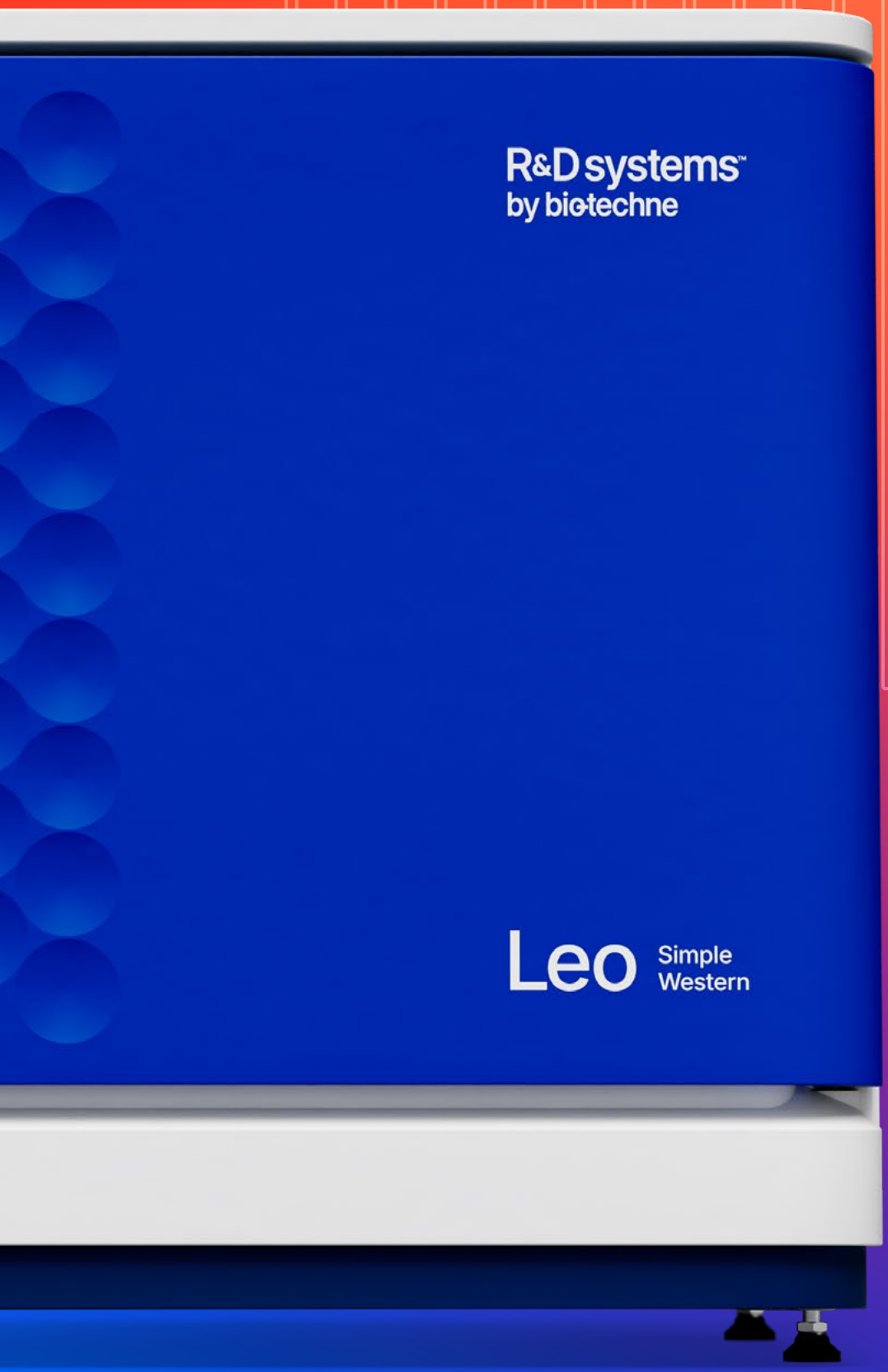
In today's fast paced research environment, traditional protein analysis methods, manual workflows, inconsistent data, and limited sensitivity slow discovery and weaken confidence in results. When precision matters and time is critical, you need more than a workaround—you need a smarter, automated solution.

Meet the Leo™ System, the next leap in Simple Western™ Technology, delivering high throughput, automated protein quantification with exceptional sensitivity and reproducibility. Leo's capillary electrophoresis immunoassays are robust bioanalytical methods suitable for use in research, translational and regulated environments.

Explore Leo with an immersive 3D experience and navigate Leo's features at your own pace in a virtual environment.

Take 3D Tour

Scan the QR code or visit
embed.envoke-demos.com/bio-techne/leo_platform/index.html



Precision & Throughput

The Leo Advantage

Accelerate Results with a High-throughput, Hand-free Workflow

Up To 100 Capillaries in as Little as 3 Hours

Save Your Precious Samples

Only 3 µl Sample/Well

Confidence in Data

With Highly Precise, Accurate and Reproducible Measurements

Detect Low Abundance Targets

Low Picogram Sensitivity in Chemiluminescence

Antibody Pairs Not Required

Save time and effort needed to find compatible antibody pairs, derisk downstream development

Maximize Data from Your Samples

With Chemiluminescence and Fluorescence Detection Channels, RePlex and Resample

Flexible Normalization Options

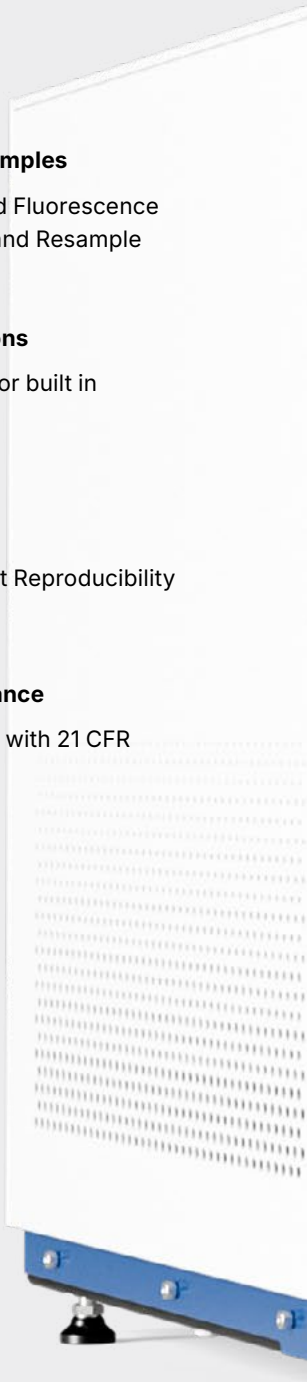
With Housekeeping Proteins or built in Total Protein Normalization

Streamline Collaboration

High Instrument to Instrument Reproducibility

Support Regulatory Compliance

Compass for Simple Western with 21 CFR Part 11 Compliance



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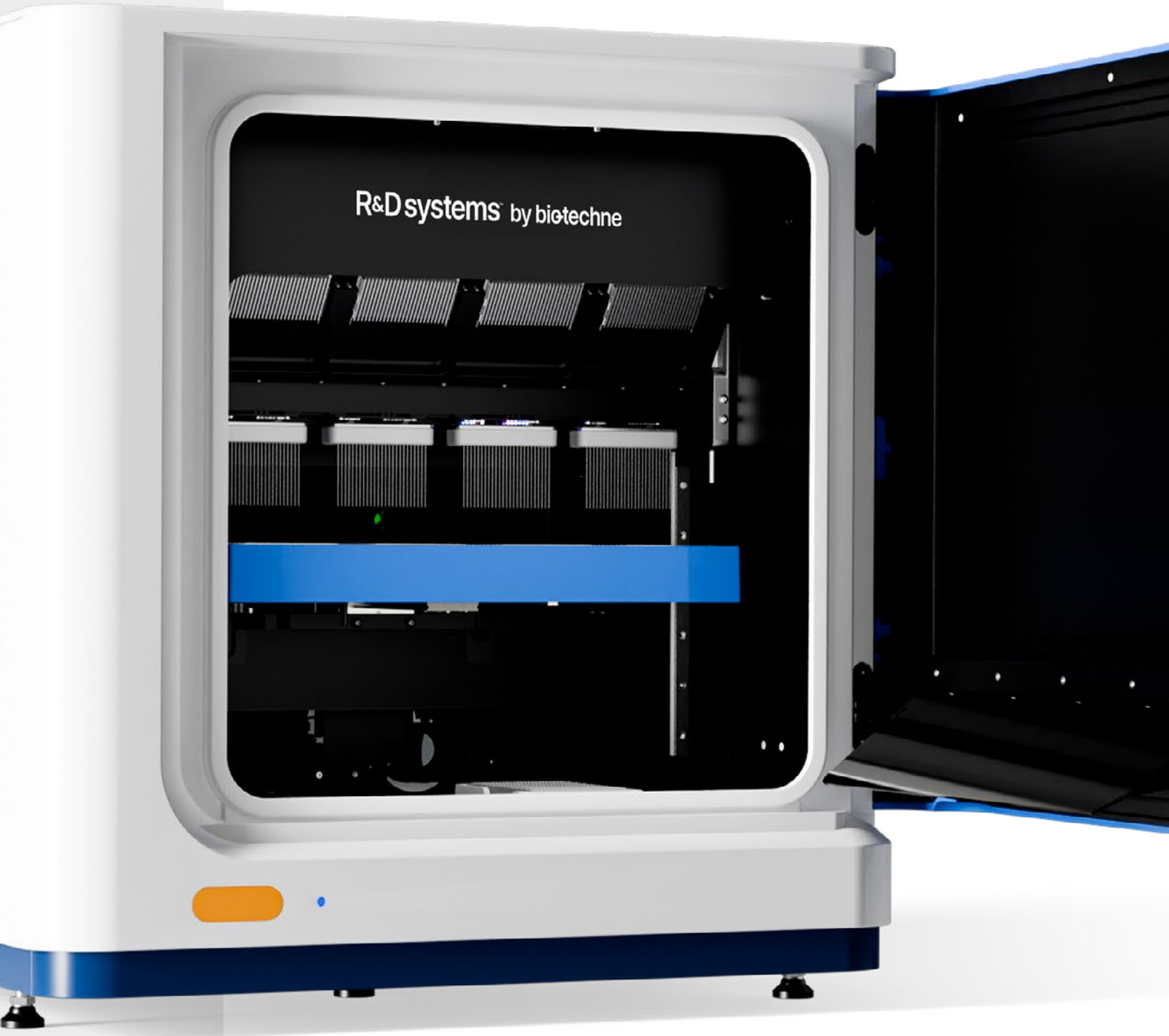
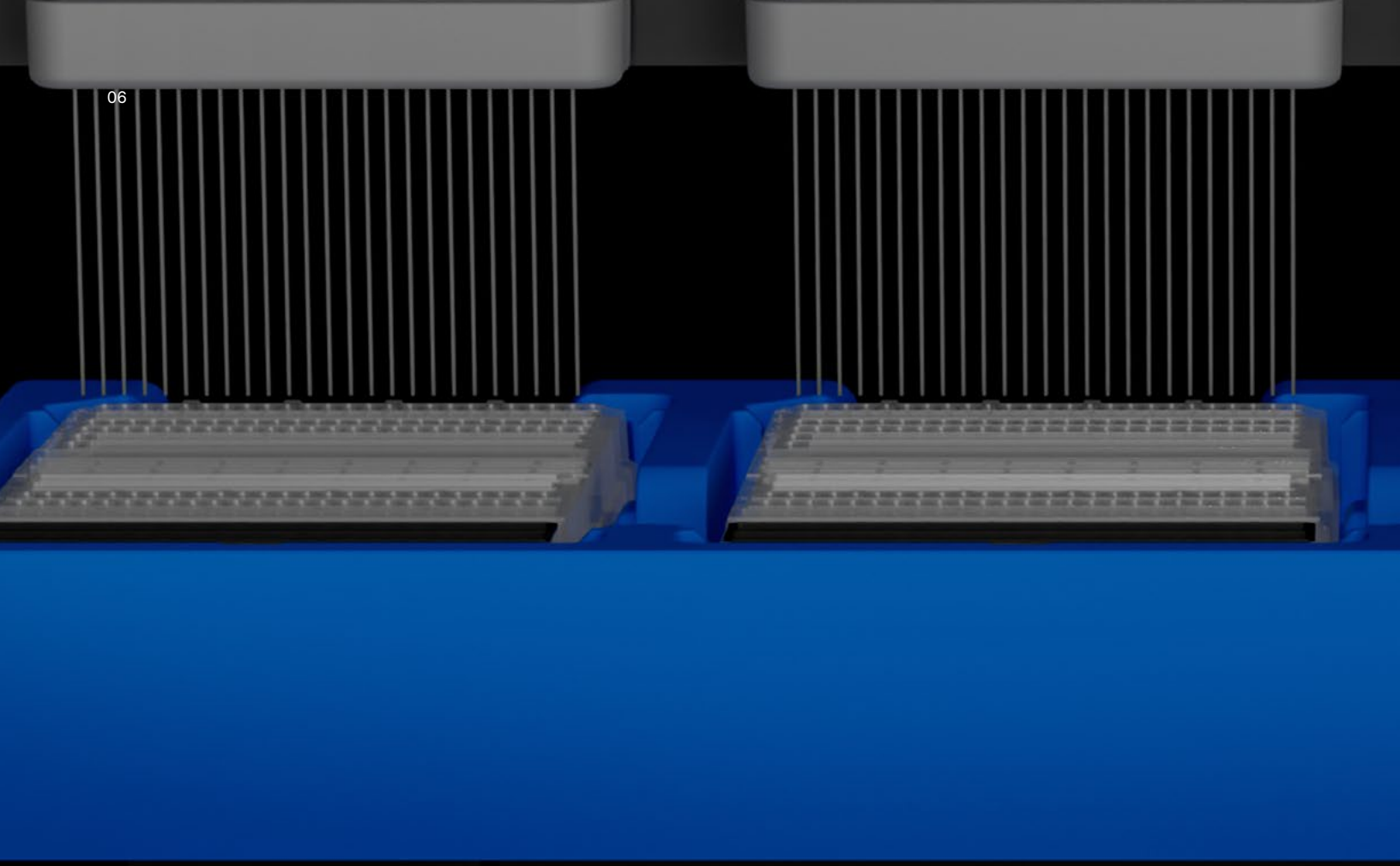

R&D systems by biotechne

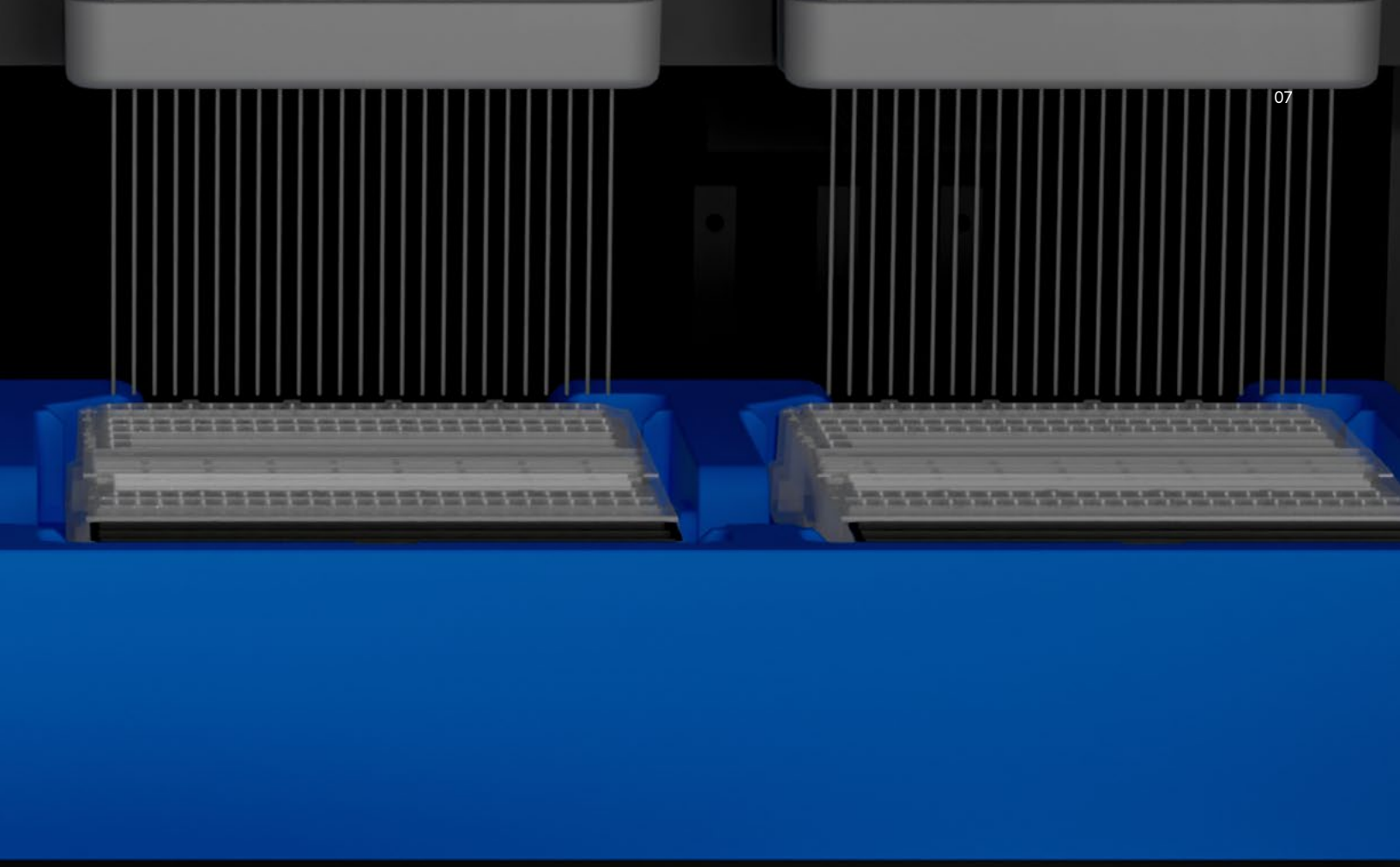


How Does Leo Work?

Leo is built on proven and trusted Simple Western technology. It separates proteins by size using capillary electrophoresis. Separated proteins are immobilized on the capillary surface and can be identified using an immunoassay. Simply load your samples and reagents into the microplate, and Leo takes care of the rest. It precisely manages every step: protein separation by size, antibody additions, incubations, washes, and even detection. Return to thoroughly analyzed, quantified results in just 3 hours.

Leo on the Inside

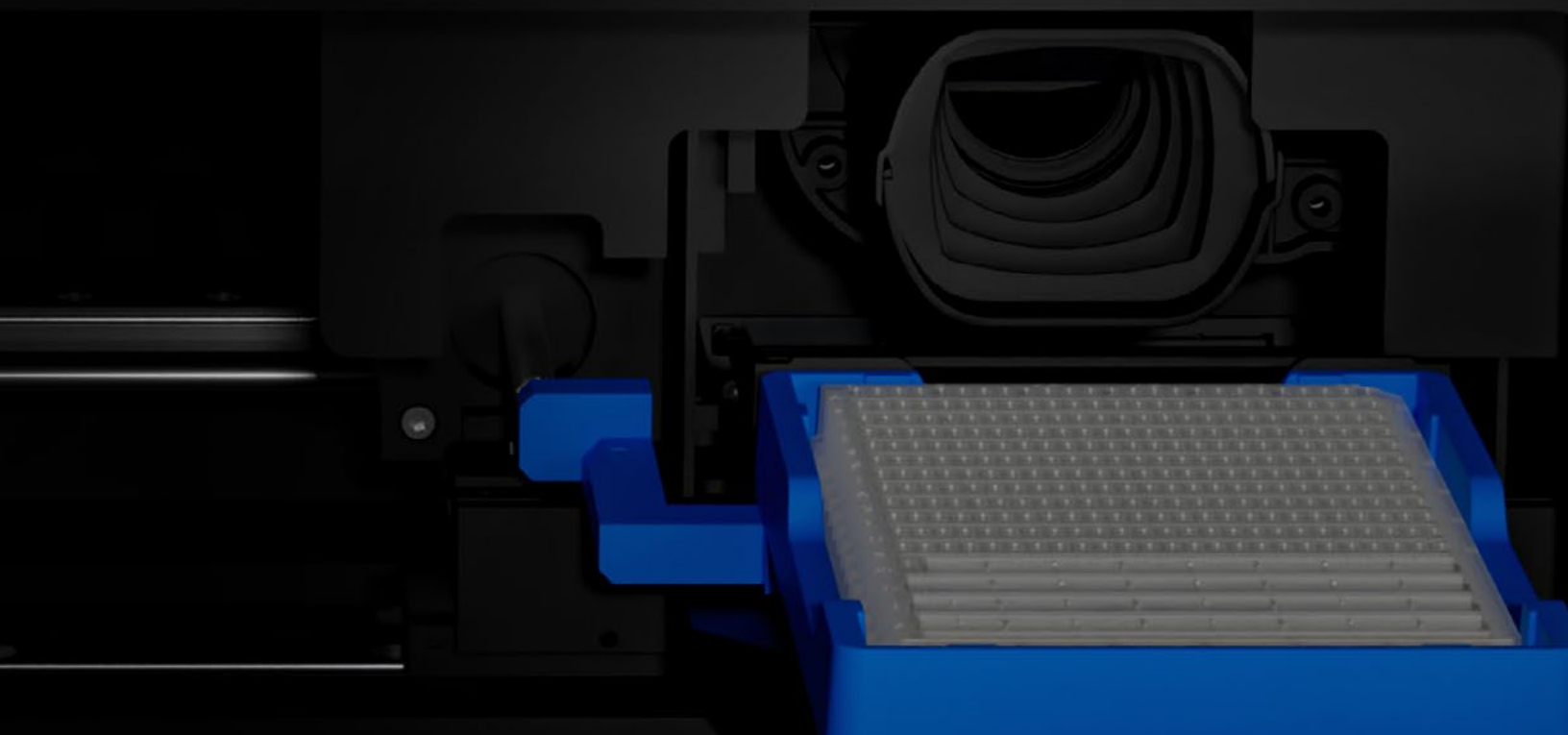
Leo holds up to 4 capillary cartridges, 4 peel and go pre-filled reagents and 1 sample plate with room for 96 samples and 4 molecular weight ladders per run. Supporting Leo is a complete set of reagents and consumables and Compass for Simple Western software. The capillary cartridges move back and forth between pre-filled reagent plates and the sample plate to load samples and reagents into the capillaries, perform protein separation, immobilization, and detection.



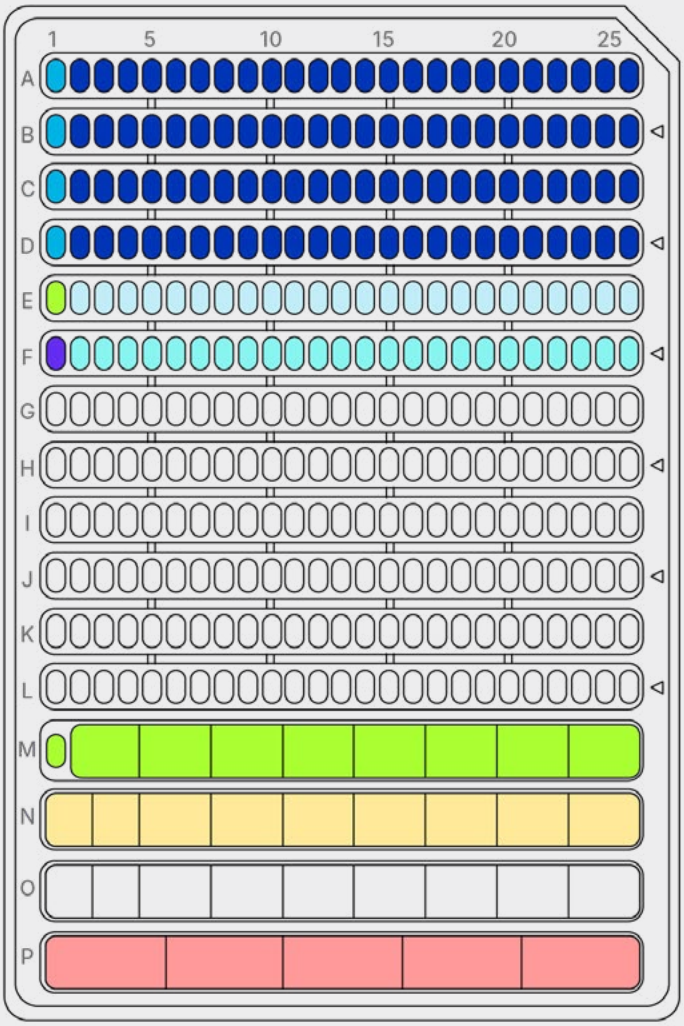
4 25-Capillary Cartridge Holders

4 Pre-filled Reagent Plants

1 Sample Plate



Leo Sample Plate



96 Samples + 4 Biotinylated Ladders

- Samples
- Biotinylated Ladder
- Antibody Diluent
- Primary Antibody
- Streptavidin-HRP or NIR
- Secondary Antibody
- Antibody Diluent
- Wash Buffer
- Luminol/Peroxide (For chemiluminescence only)

CE-ing is Believing

Watch Video to See How Leo Works

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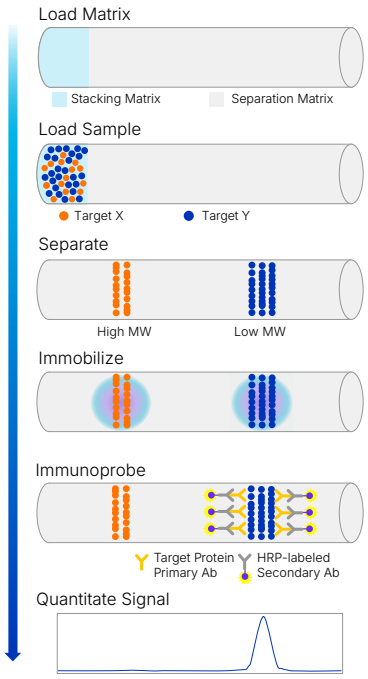
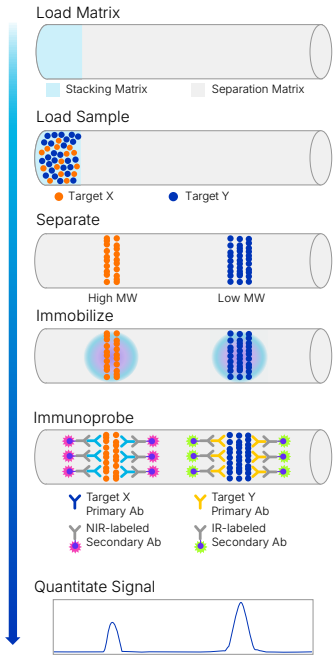
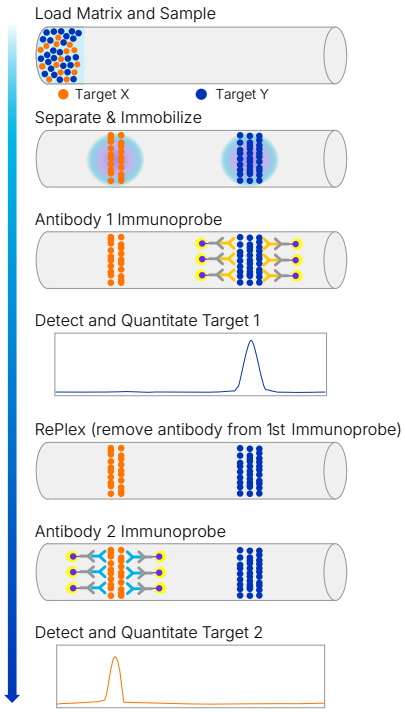
Sized-Based Assay:
Chemiluminescent Detection



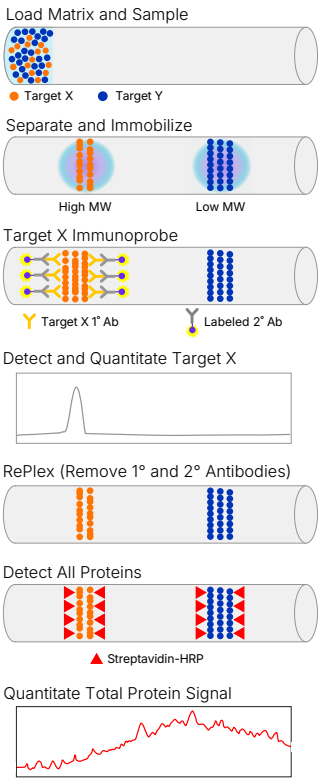
Sized-Based Assay:
Fluorescent Detection



RePlex Assay
Run Two Sequential Immunoassays



Total Protein Normalization using
RePlex and Chemiluminescence Detection



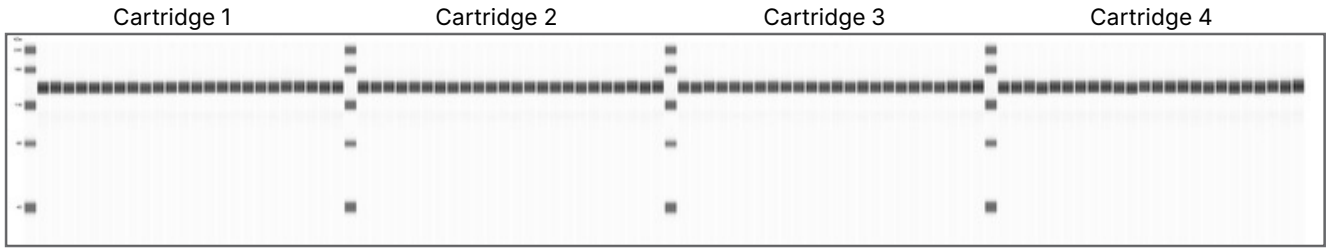
Discover the Power of Leo

Accelerate Results by Processing Up to 100 Capillaries in As Little As 3 Hours

Leo allows you to process more samples in a day, with the capacity of up to 100 capillaries in a single hand-free run.

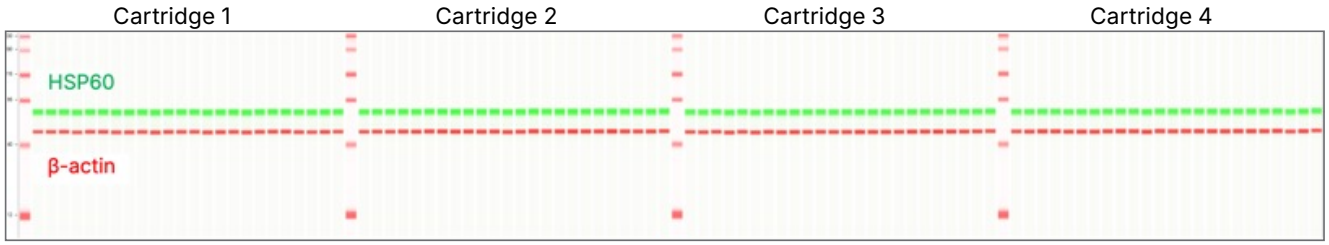
FIGURE 1

Leo Chemiluminescence immunoassay



96 samples / 4 ladders / 1 target / 3-hour run

Leo Fluorescence immunoassay



96 samples / 4 ladders / 2 targets / 5-hour run

Figure 1. Leo accommodates up to four cartridges per run, each processing 25 capillaries, for a total capacity of up to 100 capillaries in a single automated run. This configuration enables up to four molecular weight ladders and 96 unique samples per experiment. Complete run times are as little as 3 hours for chemiluminescence detection, or approximately 5 hours for fluorescence detection (NIR and IR), allowing high sample throughput within a single working day.

Large Experimental Size for Precise Quantitation

Leo can accommodate up to 96 sample experiments in a single high-precision Leo run, providing enough room for broad standard curves, replicates, calibrators, QC samples, and test samples.

Place your standard curve on any of Leo's 4 cartridges and use the remaining capillaries to quantify your samples with confidence. Recovery values within the standard 80–120% range expected for bioanalytical method validation can be obtained for well-optimized assays.

FIGURE 2

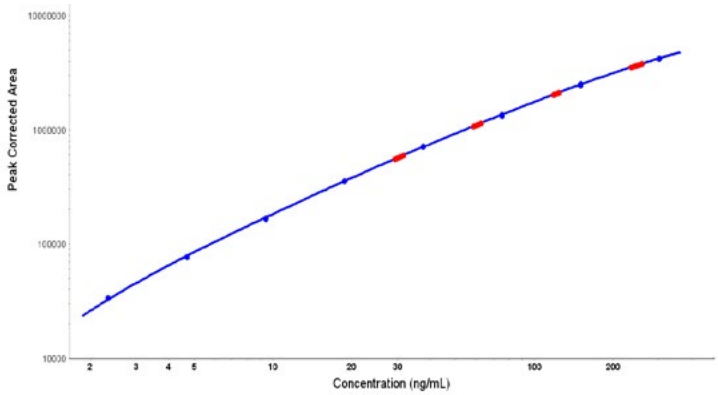


Figure 2. This plot shows an overlay of the data points for the reference samples from all four cartridges (shown in red) on the standard curve from Cartridge 1 after enabling cartridge correction. The sample data points align very well with each other, confirming that a standard curve on a single cartridge can be used for accurate and precise quantitation on Leo.

FIGURE 3A

Average % Recovery Using Intra-Cartridge Standard Curves

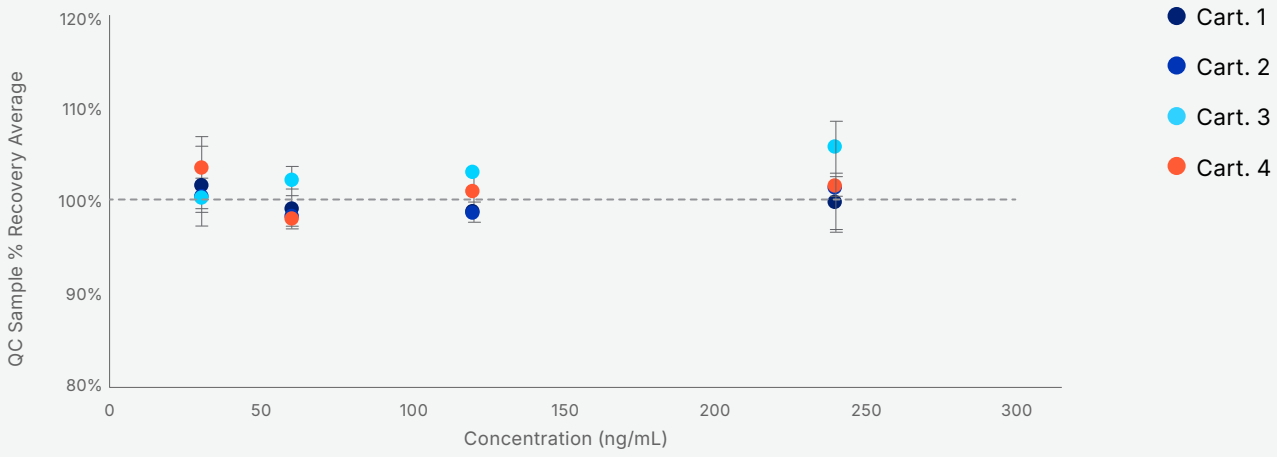


FIGURE 3B

Average % Recovery Using Standard Curve from Cartridge 1

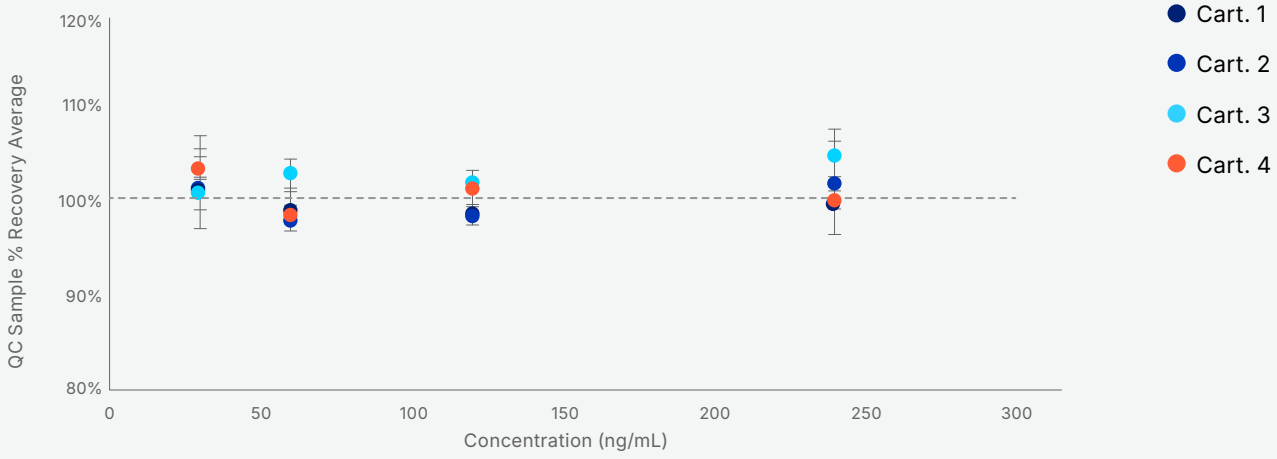


Figure 3. Spike and recovery study confirms excellent accuracy. Standard curves were defined for each cartridge independently within Compass or defined only on Cartridge 1. Then, the calculated concentrations were exported when using either the individual standard curves from each cartridge (3A) or the standard curve from Cartridge 1 (3B), and the % recovery for samples was determined by dividing Compass-calculated concentrations by known concentrations for the samples within each cartridge.

Proven Consistency for Confident Decisions

Leo offers robust and consistent performance with low CVs across cartridges within and between runs. CVs that are less than 15% for well-optimized assays underscores the high precision and reliability of Leo.

TABLE 1

Intra-Run Precision

	Cart. 1	Cart. 2	Cart. 3	Cart. 4	Intra-Run
% CV without Cartridge Correction	3.8%	5.8%	5.3%	3.7%	6.8%
% CV with Cartridge Correction	3.8%	5.8%	5.3%	3.7%	5.0%

Table 1. Cartridge correction further improves intra-assay precision. FANCD2 average peak areas across four cartridges were measured on Leo. With the new cartridge correction feature in Compass for Simple Western software, potential variations between cartridges can be normalized. Cartridge correction improved the intra-run CV to 5.0% compared to the intra-run CV of 6.8% without cartridge correction.

FIGURE 4

Leo Inter-Run Precision

Intra-Run %CV without Cartridge Correction	Intra-Run %CV with Cartridge Correction
8.0%	7.2%
6.0%	4.1%
6.8%	5.0%

Inter-Run Precision = 7.5% CV

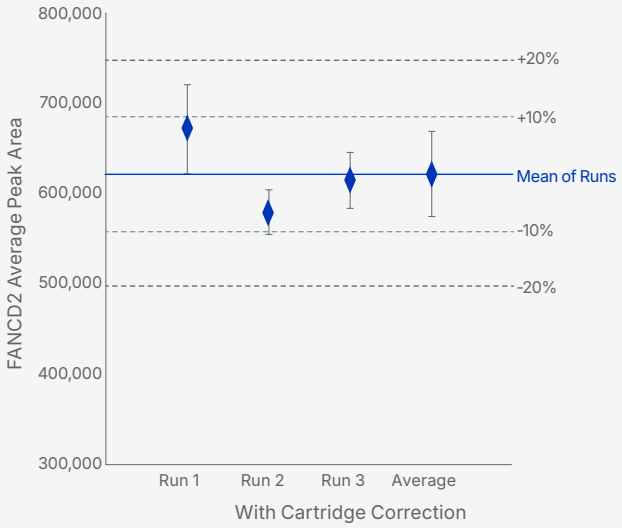


Figure 4. Three independent runs were performed on the same Leo instrument analyzing FANCD2 peak area, using split samples and matched lots of reagents, pre-filled plates, and cartridges. Even without cartridge correction, the intra-run CVs are consistently low, ranging from 6.0% to 8.0%. With cartridge correction, the CVs are further reduced, ranging from 4.1% to 7.2%. This demonstrates the inherent precision of the Leo instrument and the effectiveness of the cartridge correction algorithm. The graph on the right displays the average peak area for each run after cartridge correction, along with the overall mean and lines representing plus/minus 10% and 20% deviation from the mean of all runs. The inter-run CV is notably low at 7.5%, which indicates that the Leo system delivers highly reproducible results across multiple runs, ensuring consistency and reliability in your experiments.

Consistent Results Across Instruments for Streamlined Collaboration

Leo delivers highly consistent results across multiple instruments, ensuring data comparability and reliability regardless of the instrument used. It allows for better comparison across different locations, ensuring instrument variability does not influence results. This is particularly important for large-scale collaborative studies, clinical trials, and other applications where data consistency is paramount.

FIGURE 5

Inter-Instrument Reproducibility

Instrument	Intra-Run %CV
Leo 1	5.4%
Leo 2	5.2%
Leo 3	5.0%
Leo 4	4.9%
Leo 5	5.6%

Inter-Instrument Precision = 4.2% CV

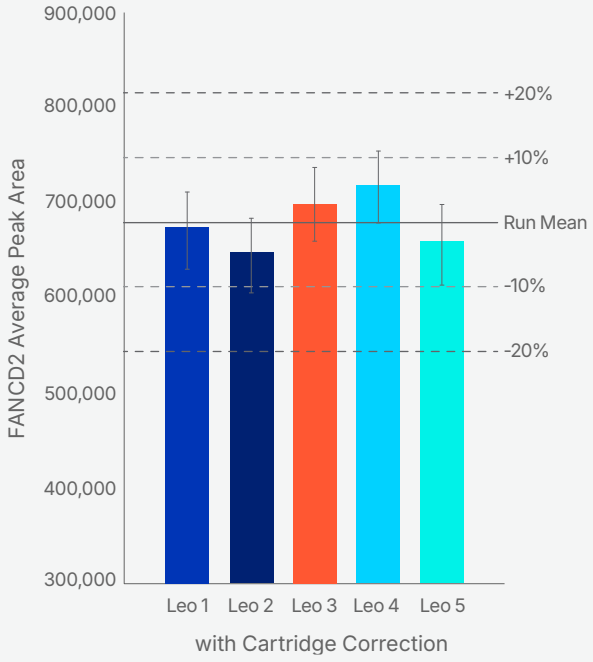


Figure 5. Inter-instrument reproducibility supporting cross-site assay transferability. For 5 Leo instruments, the average peak area of FANCD2 was measured along with the overall mean and 10% and 20% deviations from the mean. The intra-run CVs were consistently low, ranging from 4.9% to 5.6%, and the inter-instrument precision, representing the variability between the five different Leo instruments, was 4.2%. The Leo instruments were run in parallel using split samples and the same lots of cartridges, reagents, and pre-filled reagent plates.

Detect Low-Abundance Targets with Low Picogram Sensitivity

High sensitivity is essential for many applications in biological research, and Leo enables the detection of proteins down to pg/mL levels, which can be critical to measure low abundance protein biomarkers or targets for therapeutic intervention.

FIGURE 6

	Leo (pg/mL)
Chemi	253-452
NIR	883-1292
IR	3329-4949

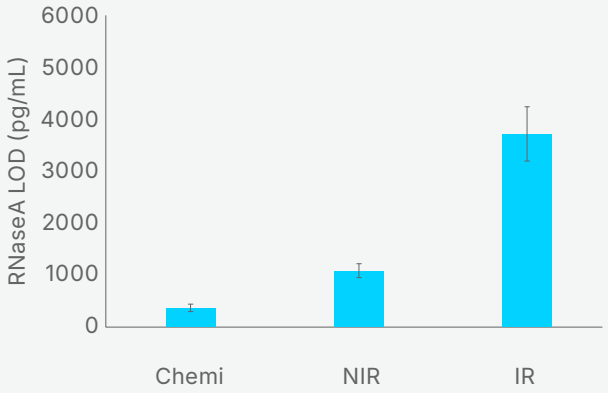


Figure 6. Leo demonstrates low hundreds of picogram to low nanogram per milliliter sensitivity, depending on the detection channel used. The low LOD values for chemiluminescence enable detection of very low abundance proteins. These results highlight Leo's strong sensitivity performance and its ability to deliver reliable LOD measurements across multiple detection channels. Note: LOD is a range for each channel because it's the LODs from two Leo runs.

Broad 3-4 Log Dynamic Range Enables Multiplexing of High and Low Signal Targets

A broad dynamic range is crucial for accurately quantifying proteins in applications where protein levels can vary significantly, such as biomarker discovery. Leo offers a broad 3-4 log dynamic range across three detection channels, which enables you to confidently quantify multiple proteins in a single sample, reducing sample preparation complexity and improving efficiency in high-throughput workflows.

FIGURE 7

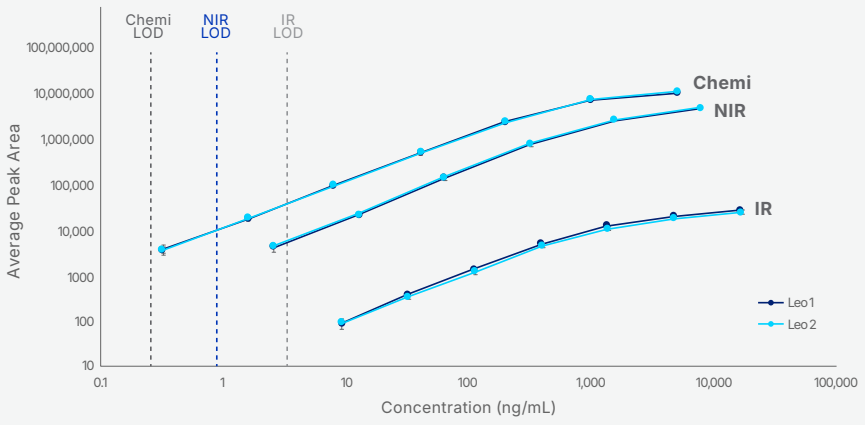


Figure 7. RNase A was detected across all three Leo detection channels—chemiluminescence, NIR, and IR. A 5-fold serial dilution series was prepared, spanning concentrations from the ug/mL range down to the low pg/mL range. Two Leo instruments were run in parallel using the same lot of reagents, plates, and cartridges. As shown in the figure, these ranges overlap significantly. Providing flexibility where high-abundance targets can be detected without saturating the signal, while low-abundance targets remain measurable without falling below the limit of detection.

Straightforward Transfer and Comparable Performance Across Simple Western Platforms

Antibodies often have quite similar EC80 values on Jess and Leo, as well as similar dynamic range and LOD.

FIGURE 8

Dynamic Range

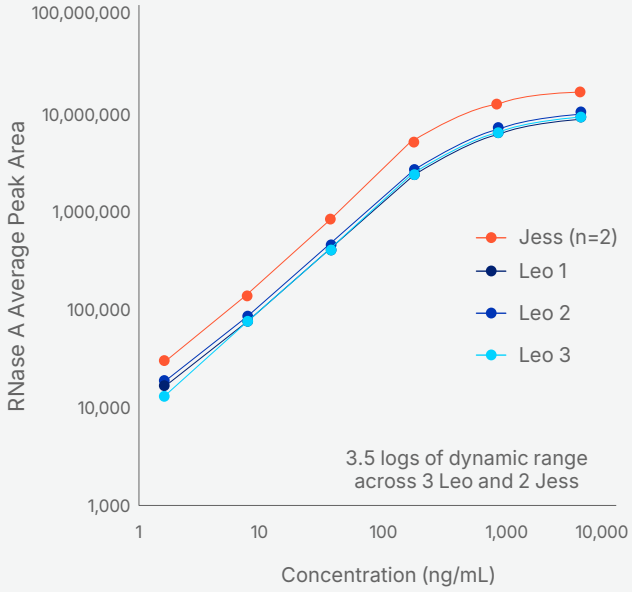


Figure 8. RNase A dynamic range and limit of detection experiments across 2 Jess and 3 Leo instruments. Both Jess and Leo produced 3.5 log dynamic ranges for RNAse A.

Maximize Data from Every Sample with RePlex and Resampling

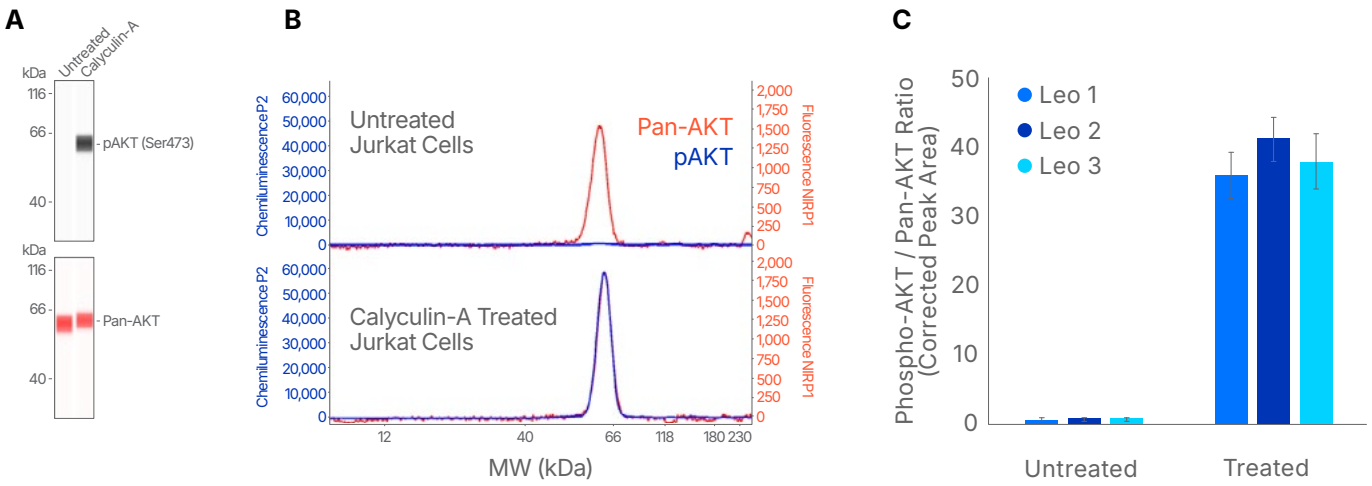
The RePlex™ assay enables two sequential immunoassays (IA) or an IA plus normalization with the Total Protein Assay (TPA) to be performed within the same capillary. Because samples are covalently bound to the capillary wall, RePlex efficiently removes antibodies between probing cycles without loss of signal intensity, preserving protein integrity and delivering reproducible, quantitative results. Addressing the challenges with traditional stripping and reprobing, RePlex gives you more data points from every sample, saves time, and confidently moves your research forward.

Resampling is another capability on Leo, which enables repeated sampling from the same wells across cartridges. Leo combines RePlex with resampling, offering a powerful multiplex option to assess up to 24 target proteins from just 3 µL of sample, all within the same run. Performing multiple analyses from a single sample reduces sample consumption and minimizes the need for repetitive sample preparation.

Alternatively, resampling can be applied to the same row of primary and secondary antibodies, reducing antibody usage by up to 50%, a savings observed when compared with previous generation assays.

FIGURE 9

RePlex enables Simultaneous Phospho / Total Detection in the Same Capillary



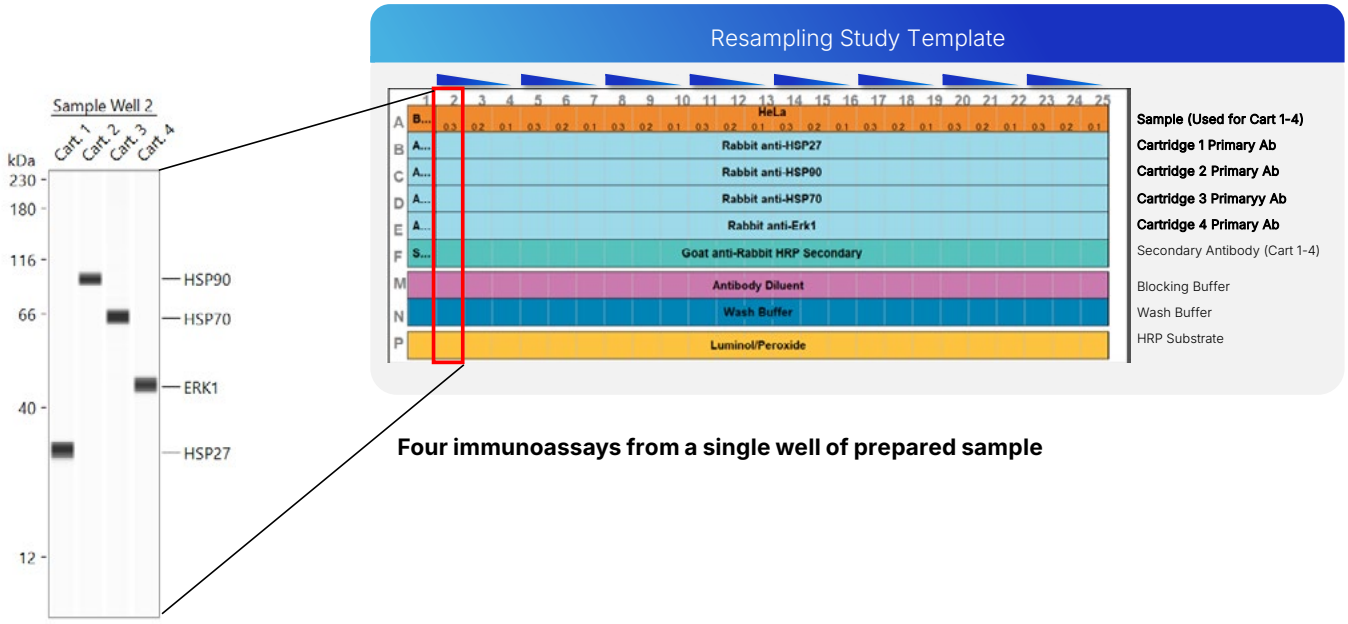
Multiplex Same Molecular Weight Targets: Detect overlapping proteins using different channels.

Broader Detection Range: Use chemiluminescence for low-abundance and fluorescence for high-abundance proteins.

Figure 9. Total and phosphorylated isoforms of the signaling protein AKT in untreated and Calyculin-A treated Jurkat cells. A) The lane view shows total AKT detected in Probe 1 and phosphorylated AKT detected in Probe 2 of RePlex, with clear phospho-AKT (pAKT) upregulation in treated cells. B) Electropherograms show how the overlapping molecular weights of total AKT and pAKT can be distinguished using two detection channels (Chemi and NIR), which simultaneously confirm the specificity of each immunoassay. C) 40- fold phospho-to-total AKT increases were observed across three Leo instruments, illustrating Leo's strong instrument to instrument reproducibility.

FIGURE 10

More Data from Each Sample Using Resampling



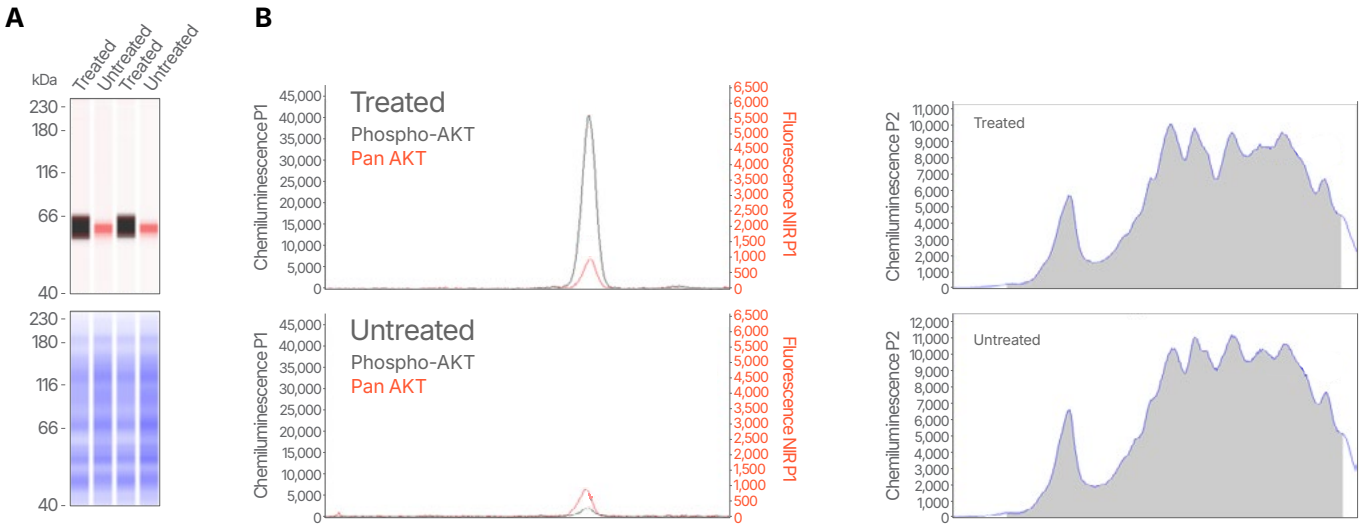
Four immunoassays from a single well of prepared sample

Figure 10. A single row of samples was loaded onto the sample plate, consisting of eight replicates each of HeLa lysates at 0.3, 0.2, and 0.1 mg/mL, for a total of 24 samples. This single sample row was used to load four cartridges within the Leo run, and each cartridge was probed with a different primary antibody. Four rows of primary antibody are shown in the plate template in the top right, with one unique row assigned to each cartridge. As with the sample row, all cartridges shared the same secondary antibody, resulting in a single row of secondary antibody. This configuration demonstrates the power of resampling, in which the same sample and the same secondary antibody are reused across multiple cartridges instead of preparing new lysates or secondary antibody for each cartridge. The results on the left display four immunoassay results for the sample in well 2, representing one immunoassay per cartridge. In this example, sample volume, preparation time, and secondary antibody volume were conserved by using resampling on Leo.

Normalization with Total Protein Using RePlex

Total protein normalization is critical for reliable protein quantification because it corrects for differences in total protein load and is not subject to biological variation that can occur with common housekeeping proteins. Normalizing immunoassay data by total protein ensures your results are both accurate and reliable. Total protein normalization on Leo is available with both chemiluminescence and fluorescence immunoassays– giving you greater flexibility, no matter the expression level of your target.

FIGURE 11



C

Sample	Primary	Cap	Name	MW (kDa)	Area	Corr. Area	Channel
Treated	rb p-SerAKT/m pan AKT	C2P1:23	Phospho-AKT	63	625519.9	750194.5	CHEMI
Untreated	rb p-SerAKT/m pan AKT	C2P1:24	Phospho-AKT	62	33951.6	35884.7	CHEMI
Treated	rb p-SerAKT/m pan AKT	C2P1:23	Pan AKT	63	13766.4	16510.2	NIR
Untreated	rb p-SerAKT/m pan AKT	C2P1:24	Pan AKT	62	13564.2	14336.5	NIR

Figure 11. Immunoassay and total protein detection performed in a single capillary. AKT phosphorylation in MCF7 lysates untreated or treated with Calyculin A. A) Phospho-AKT and pan AKT were detected in Probe 1 using chemiluminescence and NIR fluorescence, respectively, while total protein signal was detected in Probe 2. B) Example graph views of pan AKT, AKT Ser473 phosphorylation, and total protein signal for samples in panel A. C) Peaks Table in Compass for Simple Western shows automated normalization of phosphorylated and pan AKT signal to total protein signal and demonstrates quantitation of target protein expression.

Powerful Software with Advanced Analysis Features & Support for 21 CFR Part 11 Compliance

Leo comes with Compass Software for Simple Western that streamlines data analysis with an intuitive interface and robust tools, including built-in standard curve fittings and annotation features. It also enables 21 CFR Part 11 compliance to meet regulatory requirements.

Advanced Standard Curve Tools

- ✓ Compass for Simple Western has in-software tools to build a standard curve and generate reported concentrations of unknowns.
- ✓ Compass for Simple Western v7 supports the following curve fitting: Linear, 4PL, Log-Log.

Sample Group Analytics

- ✓ Group samples together by attributes defined in the Assay Layout, such as Sample Name, Primary Antibody, or more.
- ✓ Compare your replicates and get variability data quickly and easily with sample grouping.

Leo Assay Setup and Analysis

- ✓ Compass for Simple Western v7 lets you set up assays for Leo using 25–100 capillaries and 1–4 cartridges.
- ✓ Normalizes cartridge-to-cartridge variability using a reference control across each cartridge, allowing for quantitative comparisons between data from one cartridge and another.

Unleashing Analytical Applications with Leo

Leo is used in a wide variety of applications where traditional methods struggle to deliver with enough speed and quantitation. These include secondary screens of target expression during lead identification and optimization, high-throughput quantification of DC50 values for PROTACs and other targeted protein degradation compounds, relative potency assays for QC lot release for cell and gene therapy products, and measuring key biomarkers in clinical trials. Researchers also use Simple Western for diverse applications, including vaccine development, bioprocess impurity testing, pharmacokinetics/pharmacodynamics, and measuring target expression in different tissues.

FIGURE 12

Simple Western Research Area

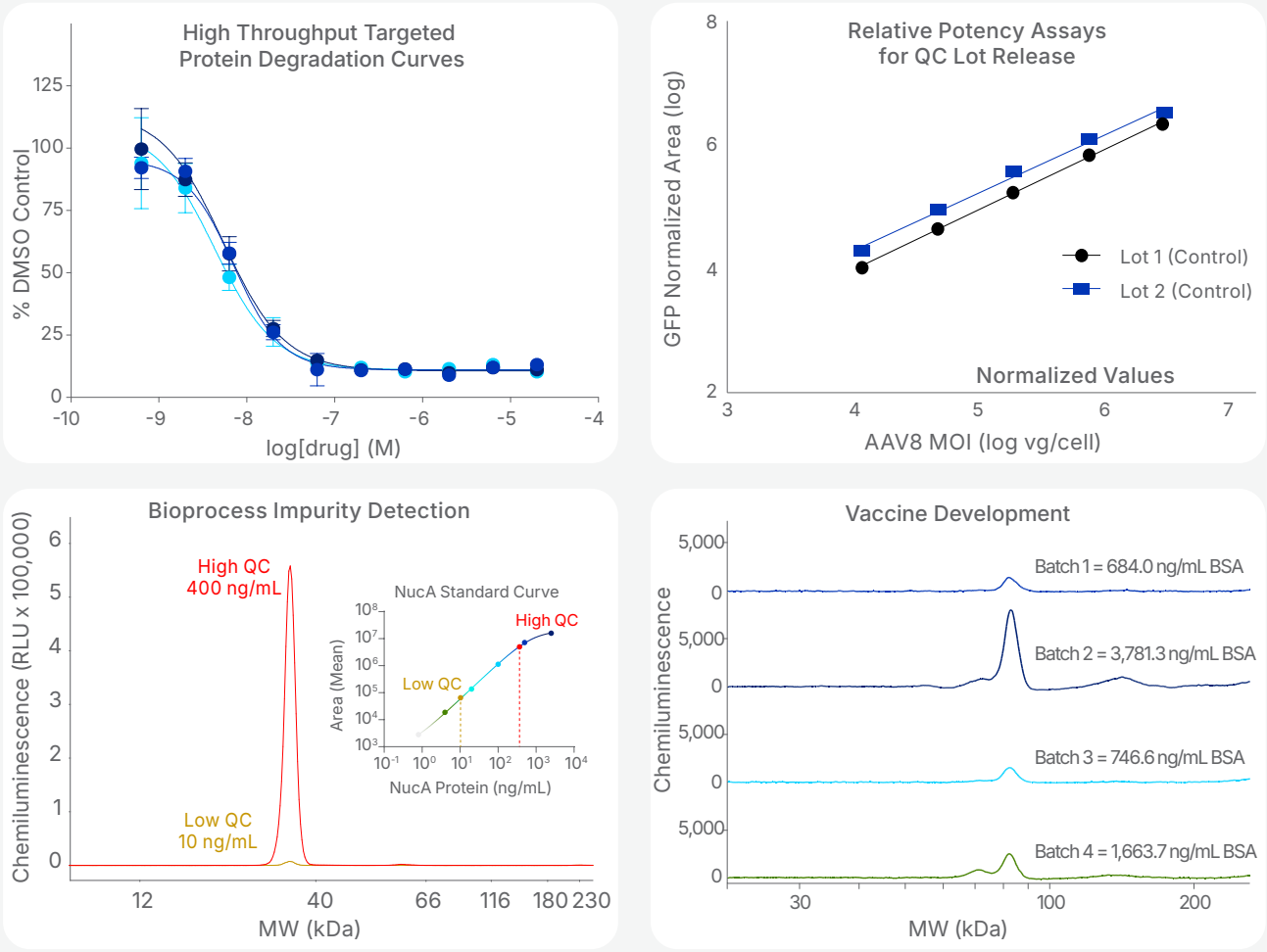
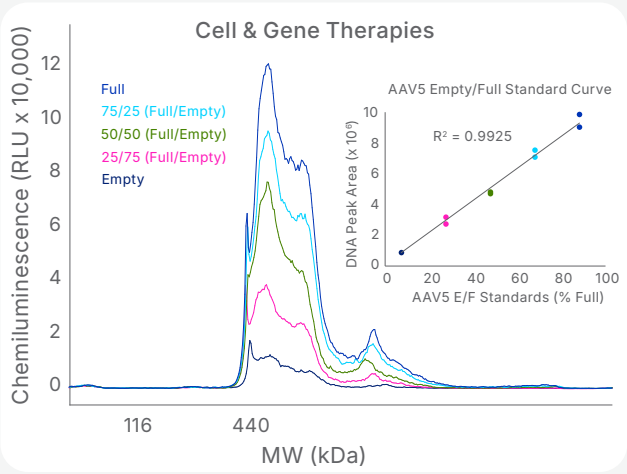
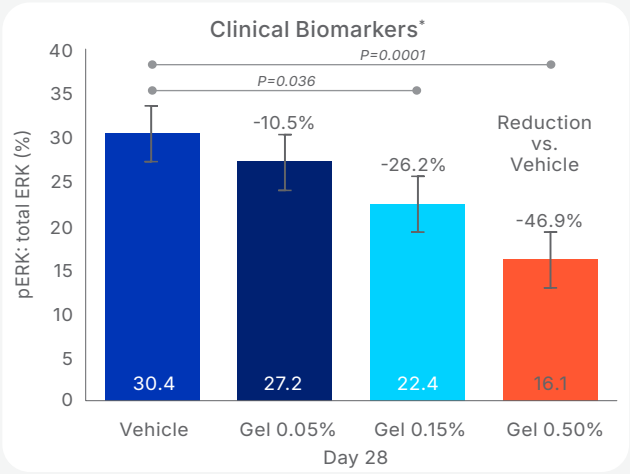
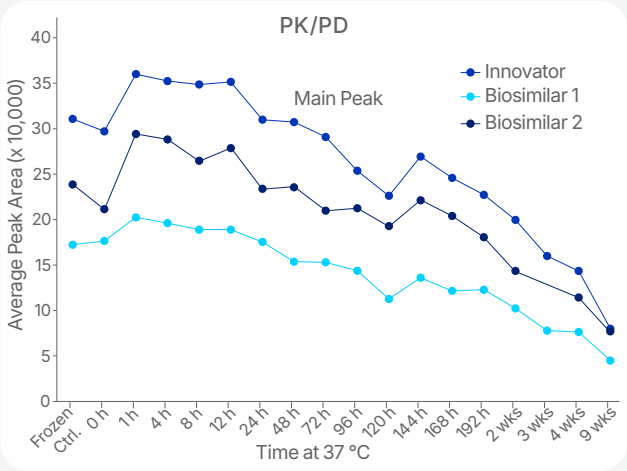


FIGURE 12

Simple Western Research Area *(continued)*







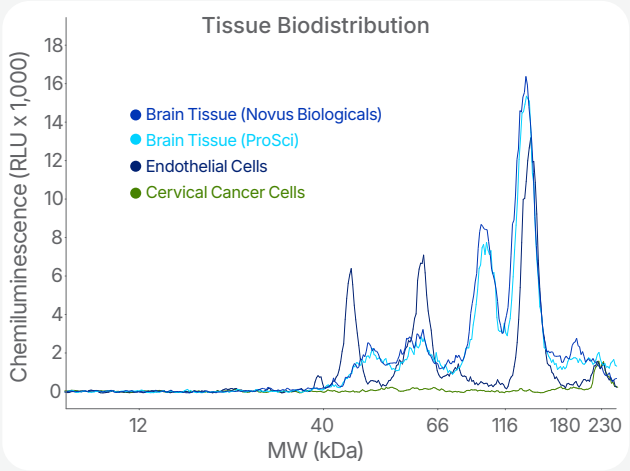


Figure 12. Simple Western Platforms have been utilized in various research areas, from cancer biology and neuroscience to drug discovery and development.
*Source: Effect of NFX-179 MEK inhibitor on cutaneous neurofibromas in persons with neurofibromatosis type 1. Sci Adv. 2024 May 3;10(18):eadk4946.

Hear From Your Peers

Alexandre Lucas, Ph.D.
Founder and Manager, We-Met at INSERM

"We chose Leo for the high throughput capacities and are getting 5-6% CVs which is quite amazing. Leo is the future for clinical research where we need absolute quantitation."

Hear Directly from Dr. Lucas

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Simple Western platforms are open systems—giving you flexibility to choose from a wide range of antibodies. Browse our curated database to find antibodies already validated for use on Simple Western.

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Leo Consumables

Access Simple Western Assay Kit Builder to find the right modules for your experiment.

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Assays and Consumables	Cat. No.
Separation Modules (For Chemiluminescence Detection)	
12-230 kDa Separation Module, 8 × 25 Caps (200 Capillaries)	**SWSM-W014**
12-230 kDa Separation Module, 24 × 25 Caps (600 Capillaries)	**SWSM-W015**
66-440 kDa Separation Module, 8 × 25 Caps (200 Capillaries)	**SWSM-W016**
66-440 kDa Separation Module, 24 × 25 Caps (600 Capillaries)	**SWSM-W017**
2-40 kDa Separation Module, 8 × 25 Caps (200 Capillaries)	**SWSM-W018**
2-40 kDa Separation Module, 24 × 25 Caps (600 Capillaries)	**SWSM-W019**
Separation Modules (For Fluorescence Detection)	
12-230 kDa Fluorescence Separation Module, 8 × 25 Caps (200 Capillaries)	**SWSM-FL014**
66-440 kDa Fluorescence Separation Module, 8 × 25 Caps (200 Capillaries)	**SWSM-FL016**
2-40 kDa Fluorescence Separation Module, 8 × 25 Caps (200 Capillaries)	**SWSM-FL018**
Detection Modules (For Chemiluminescence Detection)	
Anti-Rabbit Detection Module (200 Capillaries)	**DM-001**
Anti-Rabbit Detection Module (600 Capillaries)	**SWDM-021**
Anti-Mouse Detection Module (200 Capillaries)	**DM-002**
Anti-Mouse Detection Module (600 Capillaries)	**SWDM-022**
Anti-Goat Detection Module (200 Capillaries)	**DM-006**
Anti-Goat Detection Module (600 Capillaries)	**SWDM-026**
Anti-Human IgG Detection Module (200 Capillaries)	**DM-005**
Anti-Human IgG Detection Module (600 Capillaries)	**SWDM-025**
No secondary Detection Module (200 Capillaries)	**DM-003**
No secondary Detection Module (600 Capillaries)	**SWDM-023**
Biotin Detection Module (200 Capillaries)	**DM-004**
Biotin Detection Module (600 Capillaries)	**SWDM-024**
Detection Modules (For Fluorescence Detection)	
Anti-Rabbit NIR Detection Module	**DM-007**
Anti-Mouse NIR Detection Module	**DM-009**
Anti-Rabbit IR Detection Module	**DM-008**
Anti-Mouse IR Detection Module	**DM-010**
Additional Modules	
RePlex™ Module	**RP-001**
Total Protein Detection Module, Chemi (200 Capillaries)	**DM-TP01**
Total Protein Detection Module, Chemi (600 Capillaries)	**SWDM-TP21**

Leo Instrument Specifications

Specifications	
Runtime	~3 hours or ~ 5 hours with RePlex or Fluorescence Detection
Capillaries Per Run	Up to 100
Samples Per Run	Up to 96
Sample Volume Required	3 µL/well
Sample Cooling	No
Detection	Chemiluminescence and Fluorescence (NIR and IR)
RePlex	Yes
Weight	136 kg / 300 lbs
Dimensions (H x W x D)	0.75 m x 0.70 m x 0.73 m / 2.5′ x 2.3′ x 2.4′
Power	US/CAN 120 V AC, 60 Hz, 5.0 amps China 220 V AC, 50 Hz, 2.7 amps Europe 230 V AC, 50 Hz, 2.6 amps Japan 100 V AC, 50/60 Hz, 6.0 amps
Operating Temperature	18–23 °C
Operating Relative Humidity (non-condensing)	20–60 °C

Representative Performance Data

		Chemiluminescence Immunoassay	Total Protein with Chemiluminescence	Fluorescence Immunoassay
Precision	**Intra-assay Peak Area CV**	<15%[1]	<15%[1]	<15%[1]
	Inter-run Peak Area CV	<15%[2]	N/A	<15%[1]
	Intra-assay Quantitation CV	<10%[3]	N/A	<20%
	Inter-run Quantitation CV	<10%[3]	N/A	N/A
	Inter-instrument Quantitation CV	<10%[3]	N/A	N/A
Sensitivity	**Limit of Detection**	Low pg	ng	High pg
Dynamic Range		3-4 logs	2-3 logs	3-4 logs
Size Range		Molecular weight (MW) ladder ranges from 2-440 kDa		
Sizing CV		<10%		
Resolution (± Difference in MW)		± 10% for MW >20KDa ± 15-20% for MW <20KDa		
Sample Required		0.3-1.2 µg+	0.3-1.2 µg+	2-4 µg+

Representative performance data for well characterized assays using split samples, same lots of reagents, plates, and cartridges for each experiment.
[1]Based on model assay system with signal to noise ratio > 250, n= 96, Leo uses cartridge correction.
[2]Based on model assay system, signal to noise ratio >250, n=3, Leo uses cartridge correction.
[3]%CV of calculated concentration generated using a standard curve for all samples above LLOQ. Leo uses cartridge correction.
+ Sample required per capillary @ 3µL/capillary


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