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Product Information

SILu™MAb K1 Pharmacokinetic Kit

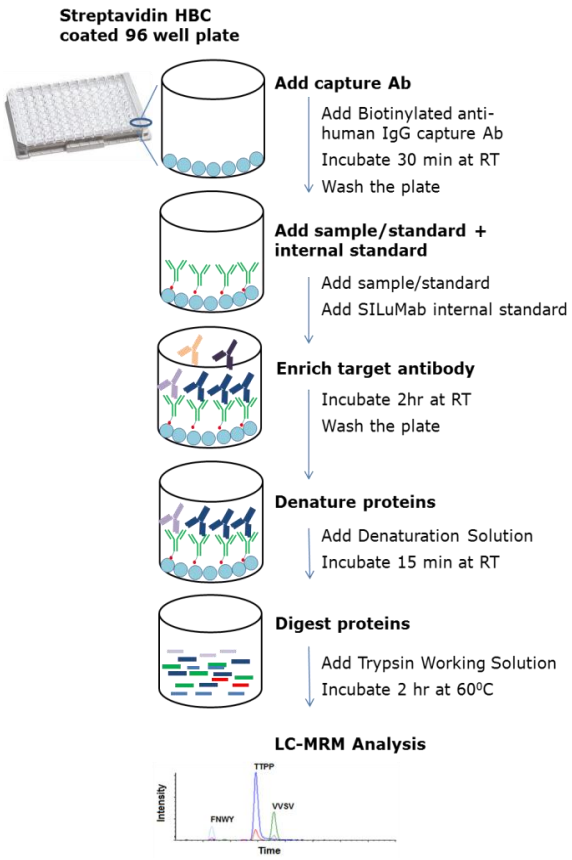
Catalog Number **MSKT0001**
Storage Temperature 2–8 °C

Product Description

The SILuMAb K1 Pharmacokinetic (PK) kit enables a robust, high-throughput assay for quantification of human IgG1 antibody in animal sera by LC-MS/MS. All critical reagents necessary to perform the assay are provided in the kit. The plate-based format avoids the high cost and potential LC column blockage associated with bead-based enrichment formats.

Sample preparation with the SILuMAb PK kit can be performed in under 5 hours and consists of coating wells with high-specificity capture antibody, followed by immunoaffinity enrichment and rapid, in-plate trypsin digestion. The resulting digest is ready for injection, with no SPE or other cleanup required.

Figure 1.
Workflow for SILuMAb PK kit



The kit includes a stable isotope-labeled SILuMab internal standard that is added to the sample early in the workflow to normalize all processing variation. The internal standard is an IgG1-kappa antibody labeled with [$^{13}C_6$, $^{15}N_4$]-Arginine and [$^{13}C_6$, $^{15}N_2$]-Lysine, and contains universally conserved IgG1 protein motifs.

Extensive validation has been performed to identify universal tryptic “surrogate peptides” that provide desirable intensity and peak shape for reliable quantification in a number of animal sera. Two monitoring and one quantitative peptide are recommended. Using the recommended peptides and analytical parameters, users can expect a linear quantification range of 0.1–12.5 μg/mL using just 5 μL of sample. Alternatively, a quantification range of 0.1–200 μg/mL can be realized by using a non-linear regression, such as 5PL regression frequently used in ELISA assays.

Components

Product Description	Catalog Number	Quantity
Streptavidin High Binding Capacity Coated Plates	S2577	1 plate
Anti-Human IgG antibody (biotinylated capture antibody)	SAB204908	1 mL
SILuMAB K1 Stable-Isotope Labeled Universal Monoclonal Antibody (internal standard)	MSQC6	100 μg
SOLu-Trypsin	EMS0004	4 × 100 μL (1 mg/mL)
MS Denaturation Solution	EMS0010	10 mL
Rapid Trypsin Digestion Buffer	EMS0009	30 mL
Tris Buffered Saline with TWEEN® 20 (TBST) powder, pH 8.0	T9039	1 packet
Tris Buffered Saline (TBS) powder, pH 8.0	T6664	1 packet
EZ-Pierce™ plate seal	Z721581	4 films

Reagents and Equipment Required but Not Provided.

- 88–91% Formic acid (Catalog Number 399388)
- LC-MS grade water (Catalog Number 1.15333)
- Acetonitrile (Catalog Number 1.00029)
- Precision single-channel pipettors certified to deliver 2 µL to 1 mL volumes
- Precision multichannel pipettors certified to deliver 5 µL to 250 µL volumes
- LC column, such as BioShell A160, 0.5 mm × 10 cm × 2.7 µm (Catalog Number 67096-U)
- Orbital shaker, such as Barnstead Thermolyne AROS 160 Adjustable Reciprocating Orbital Shaker
- Thermomixer, such as Eppendorf Thermomixer C
- LC-MS/MS system, such as Sciex 5500

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the kit at 2–8 °C. The kit is stable for two years refrigerated.

Procedures

Preparation of Reagents

TBST Solution – Add contents of one package of Catalog Number T9039 to 1 L of deionized water. Shake until fully dissolved.

TBS Solution – Add contents of one package of Catalog Number T6664 to 1 L of deionized water. Shake until fully dissolved.

Capture Antibody Solution – Add 900 µL of Capture Antibody (Catalog Number SAB204908) to 21.6 mL of TBST Solution.

Internal Standard (IS) Stock Solution – Briefly centrifuge Catalog Number MSQC6 vial before opening. Add 500 µL of 0.1% formic acid to make 200 µg/mL IS solution. Moderately vortex the sample for 20 seconds and let sit at room temperature for 15 minutes to fully dissolve. Add 10 µL of IS solution to 990 µL of ultrapure water to make 2 µg/mL IS Stock Solution. This solution may be prepared during the antibody conjugation step.

Internal Standard (IS) Working Solution – Dilute 500 µL of 2 µg/mL IS Stock Solution further by combining with 9 mL of TBS Solution to make IS Working Solution (0.1 µg/mL).

Trypsin Working Solution – Mix 400 µL of SOLu-Trypsin (Catalog Number EMS0004) with 14.6 mL of Rapid Trypsin Digestion Buffer (Catalog Number EMS0009). SOLu-Trypsin vials may be centrifuged to maximize volume recovery. This solution may be prepared during the immunoaffinity enrichment step.

Preparation of Standards and QC Samples

Researchers can create their own calibration curve within the validated linear range of 0.1–12.5 µg/mL. Table 1 shows a recommended dilution scheme for building a calibration curve through serial 1:2 dilutions, and Table 2 provides a potential dilution scheme for QC standards.

Calibrators may be prepared up to 200 µg/mL if using non-linear curve fitting. Additionally, larger volumes of QC samples can be prepared to compare assay performance between different runs.

Table 1.

Suggested Dilution for calibration standards

	Conc. (ng/mL)	Blank Plasma (µL)	Spiked (µL)	Spiking solution (µL)	Final volume (µL)
Stock	25,000	–	–	–	–
H	12,500	30	30	Stock	30
G	6,250	30	30	H	30
F	3,125	30	30	G	30
E	1,562.5	30	30	F	30
D	781.25	30	30	E	30
C	390.25	30	30	D	30
B	195.3	30	30	C	30
A	97.7	30	30	B	60

Create stock by diluting antibody of interest to 25 µg/mL

Table 2.

Suggested Dilution for QC standards

	Conc. (ng/mL)	Blank Plasma (µL)	Spiked (µL)	Spiking solution (µL)	Final volume (µL)
Stock	25,000	–	–	–	–
QC H	10,000	75	50	Stock	115
QC-M	1,000	90	10	QC-H	75
QC-L	250	75	25	QC-M	100

Create stock by diluting antibody of interest to 25 µg/mL

Assay Workflow

Conjugation of Capture Antibody to Plates

1. Add 200 μL of Capture Antibody Solution to all wells being used in the analysis.
2. Incubate for at least 30 minutes at room temperature while shaking at 150 rpm.
3. Empty well contents by inversion and gentle patting on a paper towel.
4. Wash wells once with 250 μL of TBST Solution and empty contents by inversion and gentle patting on a paper towel.

Immunoaffinity Enrichment

1. To each well, add in the following in order:
   - 95 μL of IS Working Solution (equivalent to 5 μL of IS Stock Solution [2 μg/mL]).
   - 5 μL of calibration standard, QC sample, or test sample.
2. Shake at 150 rpm at room temperature for at least 2 hours.
3. Empty well contents by inversion and gentle patting on a paper towel.
4. Wash wells once with 250 μL of TBST Solution and empty contents by inversion and gentle patting on a paper towel.
5. Wash wells twice with 250 μL of TBS Solution and empty contents by inversion and gentle patting on a paper towel.
   Note: **DO NOT use TBST Solution** in this wash step.

Trypsin digestion

1. Add 50 μL of MS Denaturation Solution to each well and shake at 150 rpm at room temperature for at least 15 minutes.
2. Add 150 μL of Trypsin Working Solution to each well (equivalent to 4 μg per well). Seal the plate and Incubate at 600 rpm at 60 °C for at least 2 hours.
   Note: **Make sure the plate is sealed tightly** to avoid evaporation.
3. Remove the seal gently.
   Note: **Take care when removing the plate seal to avoid cross-well contamination.**
4. Quench digest with 5 μL of 88–91% formic acid (Catalog Number 399388). Mix by pipetting samples up and down.
5. Reseal the plate and place in autosampler for analysis. Alternatively, digests may be transferred to low protein binding autosampler vials or multiwell plate. Alternative vials/plates should be validated prior to use, as non-specific adsorptive losses can be significant.

LC-MS/MS Analysis

1. Inject 10 μL for LC-MS/MS analysis.
2. Suggested LC parameters:

   Column: BioShell A160 0.5 mm × 10 cm × 2.7 μm
   Column Temperature: 45 °C
   Auto Sampler Temperature: 8 °C
   Flow Rate: 25 μL/min
   LC Mobile Phases: Solvent A: 99.9% H_2O, 0.1% FA
   Solvent B: 100% ACN
   Gradient:

Time	%A	%B
Initial	99.0	1.0
1.00	95.0	5.0
8.00	70.0	30.0
8.10	10.0	90.0
10.00	10.0	90.0
10.10	99.0	1.0
15.00	99.0	1.0

3. Suggested MS parameters:

MRM parameters:

Peptide Sequence	Purpose	Q1	Q3	Time (ms)	DP	CE
TTPPVLDSDGSFFLYSK.+2y15+2.light	Quantification	937.465	836.417	120	50	40
TTPPVLDSDGSFFLYSK.+2y15+2.heavy	Internal standard	941.472	840.424	120	50	40
VVSVLTVLHQDWLNGK.+3y14+2.light	Monitoring	603.340	805.438	120	75.1	30.4
VVSVLTVLHQDWLNGK.+3y14+2.heavy	Internal standard	606.012	809.446	120	75.1	30.4
FNWYVDGVEVHNAK.+2y10.light	Monitoring	839.405	1067.548	120	92.3	39.1
FNWYVDGVEVHNAK.+2y10.heavy	Internal standard	843.412	1075.562	120	92.3	39.1

Instrument parameters shown are for SCIEX 5500QTRAP equipped with a Turbo Spray Source

Results

Figure 2.
Typical MRM Chromatogram

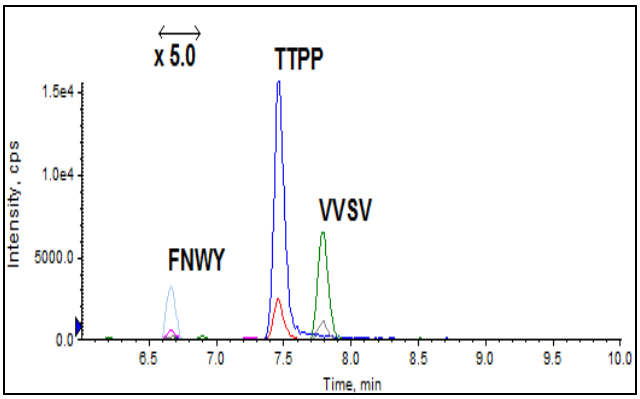
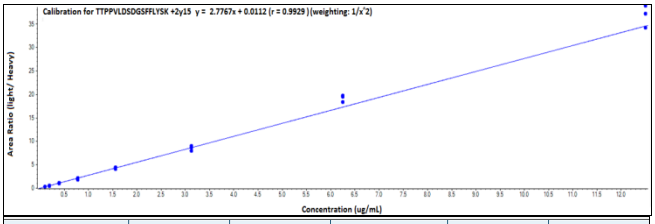


Figure 3.
Typical Calibration Curve for Infliximab in Cynomolgus Monkey Serum.



Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
0.0997	3 of 3	1.074e-1	3.586e-3	3.34	107.76
0.1953	3 of 3	1.707e-1	1.795e-2	10.52	87.39
0.3906	3 of 3	3.867e-1	2.503e-2	6.47	99.00
0.7813	3 of 3	7.095e-1	7.645e-2	10.78	90.81
1.5625	3 of 3	1.541e0	7.650e-2	4.96	98.62
3.1250	3 of 3	3.090e0	2.053e-1	6.65	98.87
6.2500	3 of 3	6.950e0	2.633e-1	3.79	111.20
12.5000	3 of 3	1.330e1	8.677e-1	6.53	106.36

The calibration data shown is linear from 0.1-12.5 µg/mL with the correlation coefficient of 0.992 and $1/x^2$ weighted curve fitting. The CV for triplicate injections are less than 15% and accuracy is ±15% at each concentration.

JK,MAM 05/18-1

Appendices
Frequently Asked Questions

1. **Can other diameter analytical columns be used?** Columns with larger or smaller diameters than the suggested 0.5 mm diameter may be used for analysis. Column loads and flow rates should be adjusted proportionally to the square of the column diameter.

2. **Can I process less than 96 samples with this kit?** The streptavidin coated plate is comprised of 8 well strips that can be processed separately. Remaining well strips can be removed from the plate frame and stored in a sealed bag with the provided desiccant. Excess reagents should be stored according to Table 3. Allow all reagents to come to room temperature before use.

Table 3.
Storage of Excess Reagents

Component	Storage
TBST Solution	2-8 °C for 1 month
TBS Solution	2-8 °C for 1 month
Capture Antibody Solution	2-8 °C for 1 month
Internal Standard Solution (at 200 μg/mL)	-20 °C for 1 month
Trypsin Working Solution	-20 °C for 1 month

3. **What animal sera have been qualified for use with this kit?** The assay is designed to work with all common preclinical animal sera, including mouse, rat, dog (Beagle), and cynomolgus monkey.

4. **Can I use this kit on human serum samples?** No, the endogenous antibodies present in human serum will interfere with capture and detection of target antibodies.

5. **Can I use this kit to quantitate IgG2 or IgG4 antibodies?** The capture and detection parameters in this kit were optimized for IgG1 antibodies and the kit has not been tested for use with IgG2 or IgG4 antibodies.

6. **Can prepared samples be stored prior to analysis?** Sample digests are stable for up to five days at 4 °C when stored in the provided assay plate. Absorptive losses may occur during storage in other vials or plates.

Troubleshooting and Tips

Problem	Possible Cause	Solution
The calibration curve is not linear	Presence of higher concentration range of target antibody	In plate based quantification the linear dynamic range is limited to 0.1–12.5 μg/mL and nonlinear range 0.1–200 μg/mL. Try plotting using different scales e.g. log-log, 5 parameter logistic curve fit, or dilute the sample to get the concentration range within 0.1–12.5 μg/mL
	Pipetting error, poor dilution series	Check pipetting technique and double-check calculations
	Adjacent well cross contamination	Make sure the seal covers the plate completely and use gentle shaking.
	Using human serum	Do not use the kit for quantification of IgG1 in human serum, the antibodies in human plasma interfere with the capture antibody. This kit made for quantification of IgG1 in animal sera preclinical applications.

The intensity of signature peptide too low	The digestion efficiency is not good	Compare the intensity of peaks with the labeled IS peptide. If both the unlabeled and labeled are not observed or have low intensity, then the problem could be digestion. Make sure the digestion buffer and denaturation buffer have pH >7. Make sure that 50 µL denaturation buffer and 150 µL of Trypsin solution are used. Double check the trypsin concentration.
	Using larger diameter LC column	Larger columns need more volume of sample on column to obtain the %CV within 20%. Inject >10 µL.
	Check that all reagents have been added in the correct order.	Double check the reagents and procedure and recalculate the sample amount.
	Washes too stringent.	For washing follow the instructions. Make sure not to touch the plate walls while pipetting
	The biotinylated antibody expired	Check the expiration dates of reagent before use
	Incorrect assay temperature (too cold)	Follow the instructions for temperature of each step
	Incorrect assay pH	Check the pH of the sample in TBS buffer before enrichment, make sure the pH is 7–8.5 before use
	Improper storage of kit	Store all reagents as recommended.
	Non-specific adsorptive losses during storage or analysis.	Sample digests should be analyzed directly in the provided assay plate when possible. If digests must be transferred to alternate vials/plate for analysis, the latter must be validated as low binding to avoid loss in sensitivity.
High variability	Pipetting	Make sure equal amounts of internal standards are used for each well. Dispense the solution into wells quickly and in the same order. Check the pipette calibration and re-run the assay and use appropriate tips.
	Cross well contamination	Make sure the seal is tight while shaking the plate. Hold the plate and gently remove the seal to avoid cross-well contamination.