

# Quantikine<sup>®</sup>

## Human SLPI Immunoassay

Catalog Number DPI00

**For the quantitative determination of human secretory leukocyte protease inhibitor (SLPI) concentrations in cell culture supernates, serum, plasma, and urine.**

*This package insert must be read in its entirety before using this product.*

**FOR RESEARCH USE ONLY.  
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

# TABLE OF CONTENTS

Contents	Page
INTRODUCTION	2
PRINCIPLE OF THE ASSAY. . . . .	3
LIMITATIONS OF THE PROCEDURE	3
REAGENTS . . . . .	4
STORAGE	4
OTHER SUPPLIES REQUIRED . . . . .	4
PRECAUTION	5
SAMPLE COLLECTION AND STORAGE . . . . .	5
SAMPLE PREPARATION	5
REAGENT PREPARATION . . . . .	6
ASSAY PROCEDURE	7
ASSAY PROCEDURE SUMMARY. . . . .	8
CALCULATION OF RESULTS	9
TYPICAL DATA . . . . .	9
TECHNICAL HINTS	10
PRECISION. . . . .	10
RECOVERY	11
SENSITIVITY . . . . .	11
CALIBRATION	11
LINEARITY . . . . .	12
SAMPLE VALUES	13
SPECIFICITY . . . . .	13
REFERENCES	14
PLATE LAYOUT . . . . .	15

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## INTRODUCTION

The secretory leukocyte protease inhibitor (SLPI) is a protein with a molecular mass of 11.7 kDa originally isolated from human parotid gland secretions (1). SLPI is a highly cationic, non-glycosylated, single-chain protein with eight intramolecular disulfide bonds (1, 2). It has been found to be a potent inhibitor of human leukocyte elastase, human cathepsin G, and human trypsin (1). SLPI has also been shown to inhibit mast cell chymase (3), a protease released during mast cell degranulation, and to inhibit histamine release from mast cells *in vitro* (4). X-ray crystallography studies have confirmed the hypothesis, based upon amino acid sequence, that the protein is composed of two homologous domains, about equal in size. Both the anti-elastase and anti-trypsin activities are mediated by the C-terminal domain. The N-terminal domain has no known function (5).

The gene for SLPI is expressed in a tissue-specific manner by cells at a variety of mucosal surfaces such as those of the lung, cervix, parotid duct, and seminal vesicles (6 - 8). In the lung, SLPI is produced in the central airways by serous glandular cells and in the lower respiratory tract by Clara cells and goblet cells (9, 10). SLPI has been shown to be one of the major proteins present in nasal epithelial lining fluid (ELF) and in nasal secretions (11 - 13).

Chronic inflammatory lung diseases such as emphysema, cystic fibrosis, idiopathic pulmonary fibrosis, and conditions resulting from cigarette smoking are characterized by increased numbers of neutrophils in pulmonary tissue and increased levels of neutrophil elastase (NE) (12, 14). NE can destroy most of the major components of pulmonary tissue and can directly injure epithelial cells and interfere with pulmonary host defenses (14). SLPI and  $\alpha_1$ -antitrypsin ( $\alpha_1$ -AT) are believed to constitute the major defenses of lung tissue against the effects of NE (14), with SLPI being the predominant defensive factor in the medium and large airways and  $\alpha_1$ -AT providing most of the protection in the alveolar region (14). It has been suggested that  $\alpha_1$ -AT is the major inhibitor of NE activity in tissues and that SLPI is the major inhibitor in secretions (11).

The measurement of SLPI concentrations in biological fluids by non-immunological methods relies on measurement of the inhibitory activity of SLPI on proteases such as chymotrypsin or trypsin (5). These measurements are rapid but not specific for SLPI. The Quantikine human SLPI Immunoassay is a 4.5 hour solid phase ELISA designed to measure SLPI in cell culture supernates, serum, plasma, and urine. It contains *E. coli*-expressed recombinant human SLPI and antibodies raised against the recombinant factor. The kit has been shown to accurately quantitate recombinant human SLPI. Results obtained using natural human SLPI showed linear curves that were parallel to the curves obtained using the recombinant kit standards. These results indicate that the Quantikine Immunoassay kit can be used to determine relative mass values for natural human SLPI.

## **PRINCIPLE OF THE ASSAY**

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for SLPI has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any SLPI present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for SLPI is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of SLPI bound in the initial step. The color development is stopped and the intensity of the color is measured.

## **LIMITATIONS OF THE PROCEDURE**

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

## REAGENTS

**SLPI Microplate** (Part 890147) - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against human SLPI.

**SLPI Conjugate** (Part 890148) - 21 mL of a polyclonal antibody against human SLPI conjugated to horseradish peroxidase, with preservatives.

**SLPI Standard** (Part 890149) - 20 ng of recombinant human SLPI in a buffered protein base with preservatives, lyophilized.

**Assay Diluent RD1Q** (Part 895079) - 11 mL of a buffered protein base with preservatives.

**Calibrator Diluent RD5T** (Part 895175) - 2 vials (21 mL/vial) of a buffered protein base with preservatives.

**Wash Buffer Concentrate** (Part 895003) - 21 mL of a 25-fold concentrated solution of buffered surfactant with preservative.

**Color Reagent A** (Part 895000) - 12.5 mL of stabilized hydrogen peroxide.

**Color Reagent B** (Part 895001) - 12.5 mL of stabilized chromogen (tetramethylbenzidine).

**Stop Solution** (Part 895032) - 6 mL of 2 N sulfuric acid.

**Plate Covers** - 4 adhesive strips.

## STORAGE

<b>Unopened Kit</b>	Store at 2 - 8° C. Do not use past kit expiration date.	
<b>Opened/ Reconstituted Reagents</b>	Diluted Wash Buffer	May be stored for up to 1 month at 2 - 8° C.*
	Stop Solution	
	Calibrator Diluent RD5T	
	Assay Diluent RD1Q	
	Conjugate	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	
	Standard	Aliquot and store for up to 1 month at ≤ -20° C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*

\*Provided this is within the expiration date of the kit.

## OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Human SLPI Controls (optional; available from R&D Systems).

## PRECAUTION

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

## SAMPLE COLLECTION AND STORAGE

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at  $\leq -20^{\circ}$  C. Avoid repeated freeze-thaw cycles.

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at  $\leq -20^{\circ}$  C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at  $\leq -20^{\circ}$  C. Avoid repeated freeze-thaw cycles.

**Urine** - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at  $\leq -20^{\circ}$  C. Avoid repeated freeze-thaw cycles. For more information on collecting and handling urine specimens, refer to the National Committee for Clinical Laboratory Standards (NCCLS) publication GP16-T.

## SAMPLE PREPARATION

All serum and plasma samples require at least a 20-fold dilution into Calibrator Diluent RD5T. A suggested 20-fold dilution is 15  $\mu$ L sample + 285  $\mu$ L Calibrator Diluent RD5T.

Urine samples require a 5-fold dilution into Calibrator Diluent RD5T. A suggested 5-fold dilution is 50  $\mu$ L sample + 200  $\mu$ L Calibrator Diluent RD5T.

## REAGENT PREPARATION

Bring all reagents to room temperature before use.

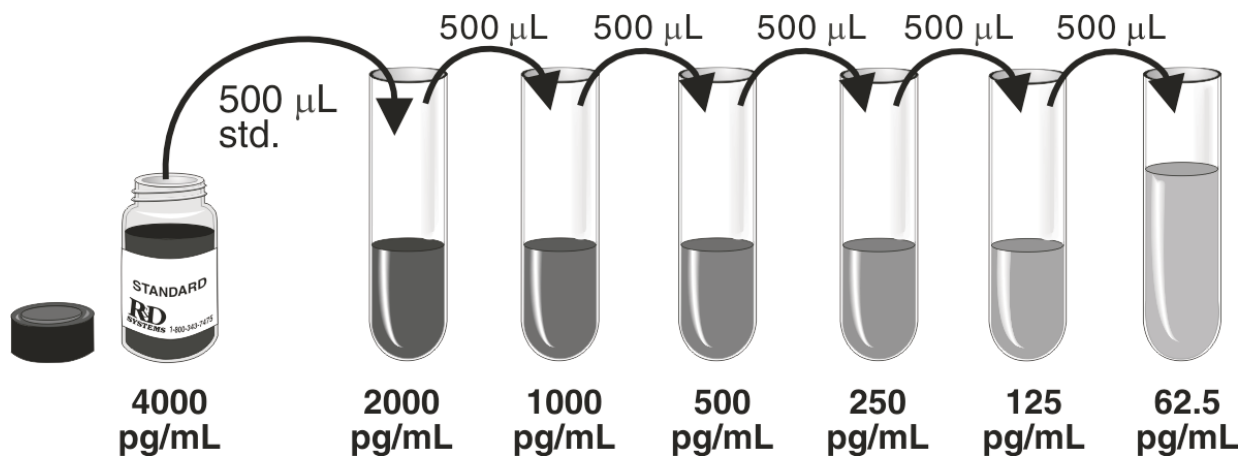
**Note:** High concentrations of SLPI are found in saliva. Take necessary precautions to protect kit reagents.

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

**Substrate Solution** - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200  $\mu$ L of the resultant mixture is required per well.

**SLPI Standard** - Reconstitute the SLPI Standard with 5.0 mL of Calibrator Diluent RD5T. This reconstitution produces a stock solution of 4000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500  $\mu$ L of Calibrator Diluent RD5T into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard serves as the high standard (4000 pg/mL). Calibrator Diluent RD5T serves as the zero standard (0 pg/mL).



## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate.**

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal.
3. Add 100  $\mu$ L of Assay Diluent RD1Q to each well.
4. Add 100  $\mu$ L of Standard, control, or sample\* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided as a record of standards and samples assayed.
5. Aspirate each well and wash, repeating the process twice for a total of three washes. Wash by filling each well with Wash Buffer (400  $\mu$ L) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper toweling.
6. Add 200  $\mu$ L of SLPI Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200  $\mu$ L of Substrate Solution to each well. Incubate for 20 minutes at room temperature. **Protect from light.**
9. Add 50  $\mu$ L of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microtiter plate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

\*Serum, plasma, and urine samples require dilution as directed in the Sample Preparation section.

## ASSAY PROCEDURE SUMMARY

1. Prepare reagents, working standards, and samples as instructed.



2. Add 100  $\mu\text{L}$  Assay Diluent RD1Q to each well.



3. Add 100  $\mu\text{L}$  Standard, control, or sample\* to each well. Incubate 2 hrs. RT



4. Aspirate and wash 3 times.



5. Add 200  $\mu\text{L}$  Conjugate to each well.  
Incubate 2 hrs. RT



6. Aspirate and wash 3 times.



7. Add 200  $\mu\text{L}$  Substrate Solution to each well.  
Incubate 20 min. RT **Protect from light.**



8. Add 50  $\mu\text{L}$  Stop Solution to each well.  
Read at 450 nm within 30 min.  
 $\lambda$  correction 540 or 570 nm

\*Serum, plasma, and urine samples require dilution.

## CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

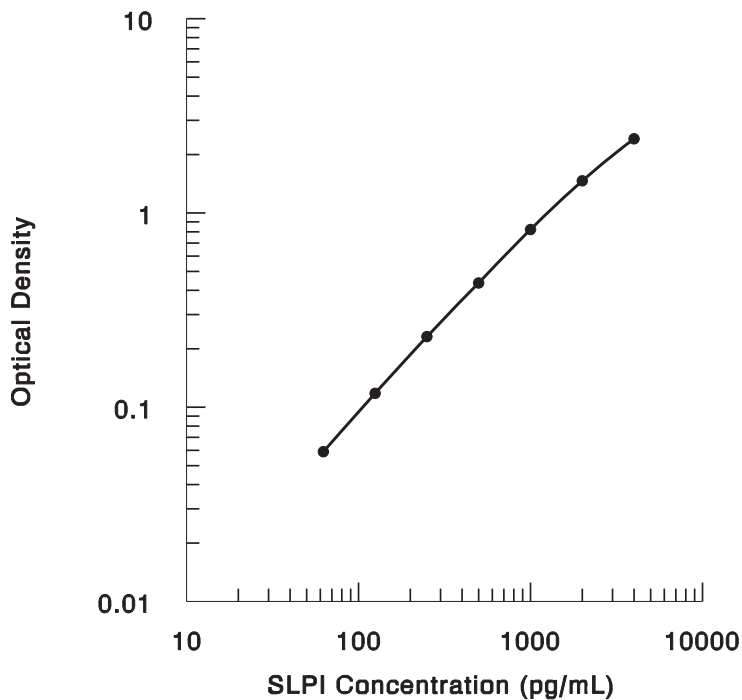
Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log/log paper and regression analysis may be applied to the log transformation.

To determine the SLPI concentration of each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding SLPI concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Because serum, plasma, and urine samples have been diluted, the measured concentrations must be multiplied by the dilution factor 20, 20, or 5, respectively.

## TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.045 0.043 0.104	0.044	—
62.5	0.102 0.163	0.103	0.059
125	0.160 0.277	0.162	0.118
250	0.273 0.480	0.275	0.231
500	0.481 0.858	0.480	0.436
1000	0.875 1.515	0.866	0.822
2000	1.503 2.486	1.509	1.465
4000	2.419	2.452	2.408

## TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

## PRECISION

### **Intra-assay Precision** (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

### **Inter-assay Precision** (Precision between assays)

Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	109	693	2026	188	1174	2157
Standard deviation	8.7	37.1	84.1	15.1	57.5	123
CV (%)	8.0	5.4	4.2	8.0	4.9	5.7

## RECOVERY

The recovery of SLPI was determined in various matrices by mixing high dose samples with low dose samples in ratios of 1:2, 1:1, and 2:1.

Sample Type	Average % Recovery	Range
Cell culture media	94	84 - 105%
Serum	98	89 - 104%
EDTA plasma	96	87 - 103%
Heparin plasma	106	98 - 116%
Citrate plasma	97	92 - 102%
Urine	98	92 - 103%

## SENSITIVITY

The minimum detectable dose of SLPI is typically less than 25 pg/mL.

The minimum detectable dose was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

## CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human SLPI produced at R&D Systems.

## LINEARITY

To assess linearity of the assay, the following biological samples containing, or spiked with high concentrations of SLPI, were diluted with Calibrator Diluent and then assayed.

Sample	Dilution	Observed (pg/mL)	Expected (pg/mL)	% <u>Observed</u> <u>Expected</u>
Cell Culture media	neat	888		
	1:2	492	444	111
	1:4	247	222	111
	1:8	112	111	101
	1:16	55	56	98
Serum*	neat	27600		
	1:2	14446	13800	105
	1:4	7082	6900	103
	1:8	3296	3450	96
	1:16	1660	1725	96
EDTA plasma*	neat	37340		
	1:2	20300	18670	109
	1:4	10244	9335	110
	1:8	4658	4668	100
	1:16	2136	2334	92
Heparin plasma*	neat	34620		
	1:2	18900	17310	109
	1:4	9824	8655	114
	1:8	4658	4328	108
	1:16	2120	2164	98
Citrate plasma*	neat	34200		
	1:2	18424	17100	108
	1:4	9470	8550	111
	1:8	4552	4275	106
	1:16	2154	2138	101
Urine*	neat	8720		
	1:2	4739	4360	109
	1:4	2383	2180	109
	1:8	1180	1090	108
	1:16	573	545	105

\* Serum, plasma, and urine preparations were initially diluted 20-fold, 20-fold, and 5-fold, respectively, in Calibrator Diluent prior to analysis as directed by the assay procedure.

## SAMPLE VALUES

**Serum/Plasma/Urine** - Forty serum, plasma, and urine samples were evaluated for the presence of SLPI in this assay.

Sample Type	Average (pg/mL)	Range (pg/mL)
Serum	36052	27380 - 46880
EDTA plasma	39647	29180 - 51160
Heparin plasma	35882	22360 - 47800
Citrate plasma	38001	26940 - 52920
Urine	4001	336 - 17425

**Cell Culture Supernates** - Human peripheral blood mononuclear cells ( $5 \times 10^6$  cells/mL) were cultured in RPMI supplemented with 5% fetal calf serum, 50  $\mu$ M  $\beta$ -mercaptoethanol, 2 mM L-glutamine, 100 units/mL penicillin, and 100  $\mu$ g/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10  $\mu$ g/mL PHA for 1 and 5 days. All samples measured less than the lowest SLPI standard, 62.5 pg/mL.

## SPECIFICITY

This assay recognizes both natural and recombinant human SLPI. Each of the factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human SLPI control were assayed for interference. No significant cross-reactivity or interference was observed.

<b>Recombinant human:</b>	IL-1ra	PDGF-AA	<b>Recombinant mouse:</b>	<b>Recombinant amphibian:</b>
ANG	IL-2	PDGF-AB	EGF	TGF- $\beta$ 5
CNTF	IL-3	PDGF-BB	IL-1 $\alpha$	
$\beta$ -ECGF	IL-4	RANTES	IL-1 $\beta$	<b>Recombinant chicken:</b>
EGF	IL-5	SCF	IL-2	TGF- $\beta$ 3
Epo	IL-6	TGF- $\alpha$	IL-3	
FGF acidic	IL-6 sR	TGF- $\beta$ 1	IL-4	<b>Other:</b>
FGF basic	IL-7	TGF- $\beta$ 2	IL-5	bovine FGF acidic
FGF-4	IL-8	TGF- $\beta$ 3	IL-6	bovine FGF basic
G-CSF	IL-9	TNF- $\alpha$	IL-7	human PDGF
GM-CSF	IL-10	TNF- $\beta$	IL-9	human TGF- $\beta$ 1
GRO $\alpha$	IL-11	sTNF RI	MIP-1 $\alpha$	porcine TGF- $\beta$ 1
IFN- $\gamma$	LIF	sTNF RII	MIP-1 $\beta$	porcine TGF- $\beta$ 2
IGF-I	M-CSF		SCF	porcine TGF- $\beta$ 1.2
IGF-II	MCP-1		TNF- $\alpha$	
IL-1 $\alpha$	MIP-1 $\alpha$			
IL-1 $\beta$	MIP-1 $\beta$			
	OSM			

Leukocyte elastase, an enzyme known to be inhibited by SLPI, was tested for cross-reactivity and interference at 250 ng/mL and 1  $\mu$ g/mL. No cross-reactivity or interference was observed. No cross-reactivity or interference from bovine pancreatic trypsin (1  $\mu$ g/mL) or chymotrypsin (500 ng/mL) was observed.

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# PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.

1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
	A	B	C	D	E	F	G	H