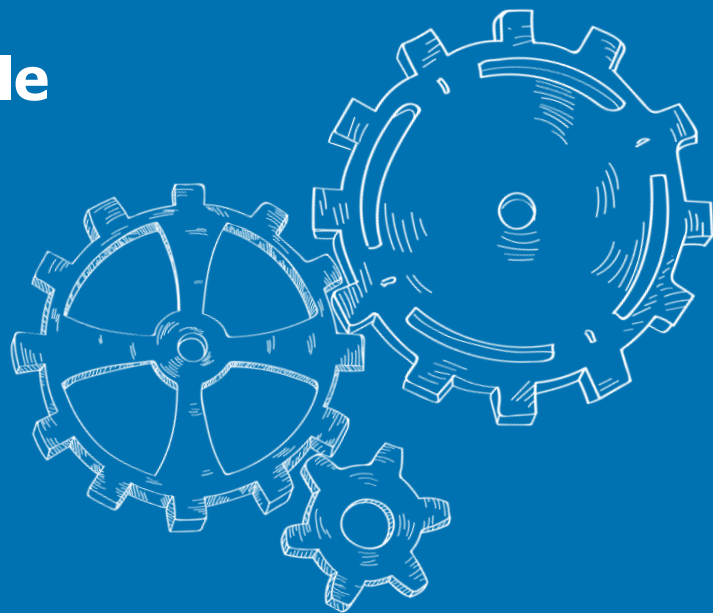


## Simple Plex™ 方法验证

ProteinSimple



# Ella 验证准则

## Linearity

DILUTION	PARAMETER	SERUM (n=4)	EDTA PLASMA (n=4)	HEPARIN PLASMA (n=4)
1:2	Avg % of Expected	105	104	94
	Range (%)	97-115	95-112	92-101
1:4	Avg % of Expected	111	109	113
	Range (%)	103-125	102-119	109-118
1:8	Avg % of Expected	105	110	113
	Range (%)	89-125	101-123	105-119
1:16	Avg % of Expected	101	109	116
	Range (%)	94-115	101-118	106-122

## ELISA correlated

That the assay has been correlated to Quantikine® ELISA Kit with a slope of 0.9-1.1 and an R<sup>2</sup> value greater than 0.9

## Precision

PARAMETER	LOW QC	HIGH QC
Intra-Mean (pg/ml)	17.8	785
Intra-assay SD	0.680	56.1
Intra-assay CV (%)	3.8	7.1
Inter-Mean (pg/ml)	17.2	744
Inter-assay SD	1.23	46.3
Inter-assay CV (%)	7.1	6.2

## Recovery

SAMPLE TYPE	AVG %	RANGE %
Serum (n=4)	95	82-106
EDTA Plasma (n=4)	91	82-100
Heparin Plasma (n=4)	86	75-94

## Validated

## Endogenous

SAMPLE TYPE	MEAN (pg/ml)	RANGE (pg/ml)	STD DEV (pg/ml)
Serum (n=10)	172	93.3-259	73.1
EDTA Plasma (n=10)	19.8	16.4-29.9	3.89
Heparin Plasma (n=10)	21.8	11.5-29.1	6.68

## Specificity

### Specificity

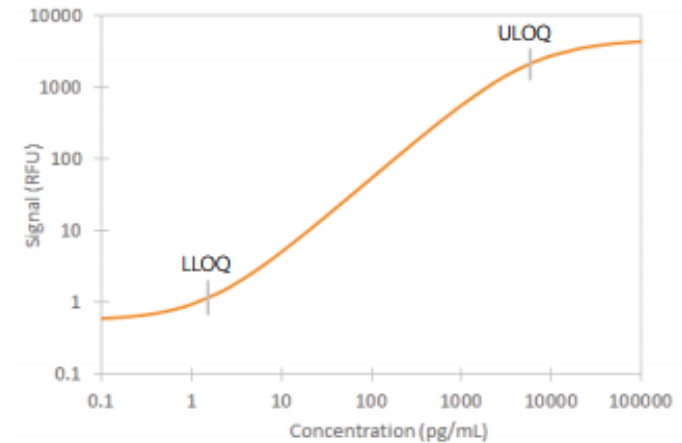
This assay recognizes natural and recombinant human IL-6. The factors listed below were assayed for cross-reactivity and interference. No significant cross-reactivity or interference was observed.

### Recombinant human:

- IL-6 Ra
- IL-10 Ra
- IL-10 Rβ
- IL-12/23 p40
- IL-12 Rβ1
- IL-12 Rβ2
- IL-18 Ra
- IL-18 Rβ
- IL-18BPα
- IL-23

# 出厂标准曲线

1. 必须由**2个用户**使用**2种不同批次**的试剂来运行至少**3个独立的标准曲线**(例如稀释液, GNR 捕获抗体, 生物素标记的抗体)
2. 采用5 PL 函数进行曲线浓度 (pg/mL) 和信号值 (RFU)的拟合
3. LLOQ (定量下限) = 计算浓度的变异系数 (CV)≤20%, 且加标回收回收率在已知值的80%-120%以内时的最低浓度
4. ULOQ (定量上限) = 计算浓度的变异系数 ( CV)≤10%, 且加标回收回收率在已知值的80%-120%以内时的最高浓度
5. LOD (检测极限)是通过在多次运行中确定的平均背景信号增加三个标准差来计算的



## Limit of Quantitation

Data shown represents typical performance results for Lower Limit of Quantitation (LLOQ) and Upper Limit of Quantitation (ULOQ) of MCP-1.

	CONC. (pg/mL)
LLOQ	1.53
ULOQ	5780

## Limit of Detection

The limit of detection (LOD) of human MCP-1 is 0.35 pg/mL. The LOD was calculated by adding three standard deviations to the mean background signal determined from multiple runs.

# 精密度

1. 采用重组对照(低浓度质控标准品和高浓度质控标准品)
2. 分析内- 1个卡盒, 16重复,  $CV \leq 10\%$
3. 分析间- 多个卡盒,  $CV \leq 15\%$
4. 多个用户- 至少3人



PARAMETER	LOW QC	HIGH QC
Intra-Mean (pg/mL)	33.2	1746
Intra-assay SD	1.19	124
Intra-assay CV (%)	3.6	7.1
Inter-Mean (pg/mL)	32.3	1744
Inter-assay SD	2.05	136
Inter-assay CV (%)	6.3	7.8

# 回收率

1. unspiked的样品 + 3个spiked浓度的4个独立的血清， 8个独立的血浆（4个EDTA， 4个肝素）， 8个独立的血小板匮乏的血浆
2. 回收率百分比:  $(\text{测定浓度} - \text{内源浓度}) / \text{对照品实际浓度} * 100$
3. 所有样本在不同浓度下的平均回收率在 75-125%之间
4. 每个样品在不同浓度下的平均回收率在70-130% 之间



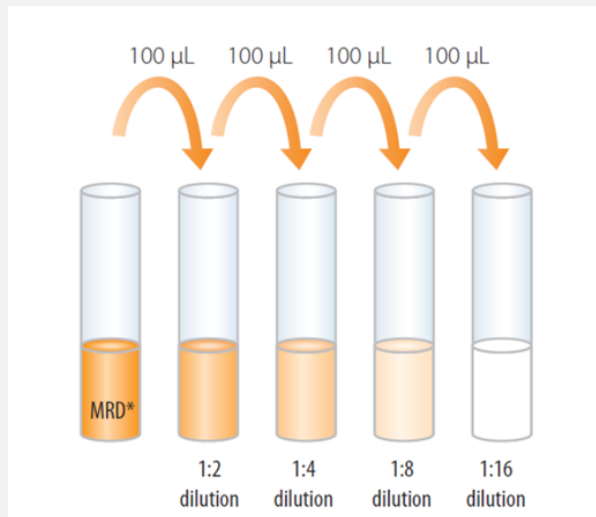
## Recovery

Recovery at three different spiked concentrations within the range of the assay was evaluated.

SAMPLE TYPE	AVG %	RANGE %
Serum (n=4)	91	82-102
EDTA Plasma (n=4)	89	79-100
Heparin Plasma (n=4)	87	80-103

# 线性

1. 至少检测1:2、1:4、1:8和1:16连续稀释梯度的4个血清、4个EDTA处理的血浆、4个肝素处理的血浆、和4个细胞上清样品
2. 天然样本或者spiked样本
3. 必须在动态范围内(LLOQ – ULOQ)
4. 在定量上下限之间的大于最小稀释量的所有稀释比例的回收率在75-125%之间



DILUTION	PARAMETER	CCS (n=4)	SERUM (n=4)	EDTA PLASMA (n=4)	HEPARIN PLASMA (n=4)
1:2	Avg % of Expected	103	94	95	100
	Range (%)	95-117	92-96	78-111	92-105
1:4	Avg % of Expected	97	99	101	100
	Range (%)	85-116	96-104	96-106	95-105
1:8	Avg % of Expected	91	103	105	107
	Range (%)	78-107	100-106	101-111	99-111
1:16	Avg % of Expected	88	107	111	107
	Range (%)	74-102	103-110	101-118	94-115

# 内样样本

1. 来自明显健康的志愿者的多个血清和血浆样本进行内源水平的筛选

## Endogenous Levels

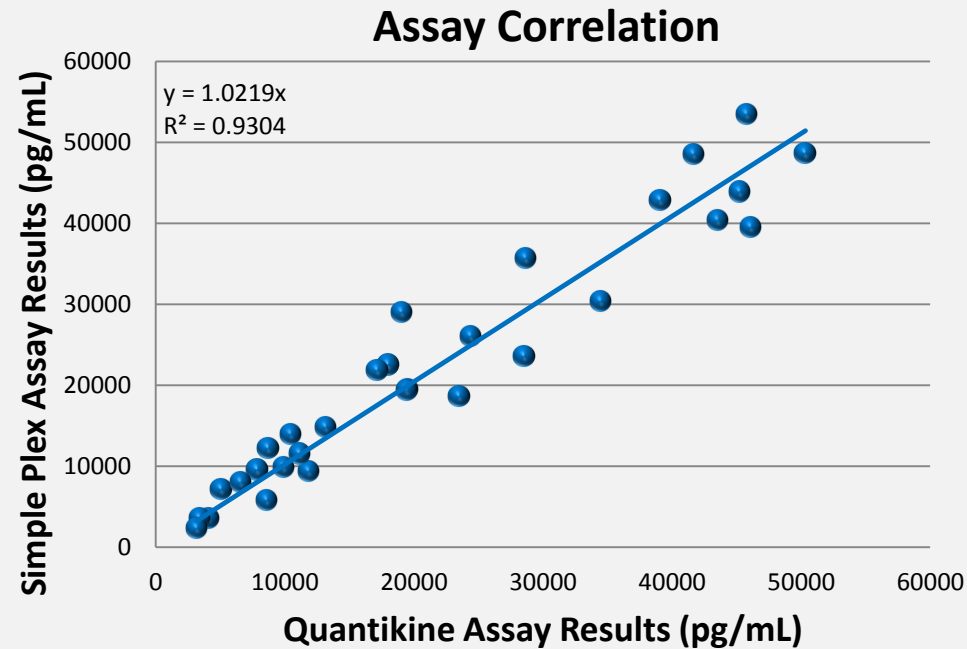
Endogenous levels were calculated from multiple samples. Samples were from apparently healthy volunteers. No medical histories were available for the donors in this study.

SAMPLE TYPE	MEAN (pg/mL)	RANGE (pg/mL)	STD DEV (pg/mL)
Serum (n=10)	296	163-485	90.8
EDTA Plasma (n=10)	175	106-429	102
Heparin Plasma (n=10)	221	157-459	89.6



# 相关性

1. 对多个样本进行分析，以比较Ella和Quantikine® ELISA Kits两个分析方法的重叠动态范围
2. 斜率在0.9-1.1之间
3. R2大于0.9



## Correlation

This assay has been correlated to the Quantikine® ELISA Kit with an  $R^2$  value greater than 0.9.

# 特异性

## 1. 对一个物种的其他类似分析物

评估交叉反应或干扰

- ① 类似分析物
- ② 可溶性受体
- ③ 可溶性结合蛋白

## 2. 类似物的检测浓度需高于定量

上限(一般为10倍)

### Specificity

This assay recognizes natural and recombinant human MCP-1. The factors listed were prepared at 34 ng/mL in Sample Diluent and assayed for cross-reactivity. Preparations of the following factors at 34 ng/mL in a rhMCP-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

#### Recombinant human:

- GRO $\alpha$
- GRO $\beta$
- GRO $\gamma$
- IL-8
- MCP-2
- MCP-3
- MCP-4
- MIP-1 $\alpha$
- MIP-1 $\beta$
- RANTES

# 验证说明书

specification sheet

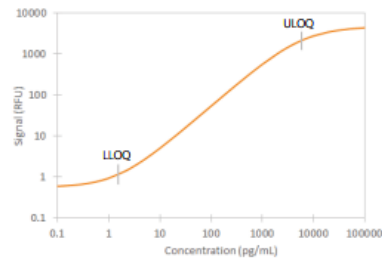
## Human CCL2/MCP-1, 2nd Generation

### Simple Plex assay for the detection of human Monocyte Chemotactic Protein 1 (MCP-1) in cell culture supernatant (CCS), serum, and plasma (EDTA/Heparin)

For research use only. Not for use in diagnostic procedures.

#### Calibration Curve

The factory generated calibration curve shown below was compiled by averaging 5 replicates of each calibrator from multiple runs. The 5PL curve fit shows calibrator concentration as a function of signal intensity (relative fluorescent units, RFU).



#### Limit of Quantitation

Data shown represents typical performance results for Lower Limit of Quantitation (LLOQ) and Upper Limit of Quantitation (ULOQ) of MCP-1.

	CONC. (pg/mL)
LLOQ	1.53
ULOQ	5780

#### Limit of Detection

The limit of detection (LOD) of human MCP-1 is 0.35 pg/mL. The LOD was calculated by adding three standard deviations to the mean background signal determined from multiple runs.

#### Endogenous Levels

Endogenous levels were calculated from multiple samples. Samples were from apparently healthy volunteers. No medical histories were available for the donors in this study.

SAMPLE TYPE	MEAN (pg/mL)	RANGE (pg/mL)	STD DEV (pg/mL)
Serum (n=10)	296	163-485	90.8
EDTA Plasma (n=10)	175	106-429	102
Heparin Plasma (n=10)	221	157-459	89.6

#### Precision

**Intra-assay Precision:** Each control was tested 16 times in one assay.

**Inter-assay Precision:** Replicates of each control were tested in multiple assays performed by at least three technicians using two lots of reagents.

PARAMETER	LOW QC	HIGH QC
Intra-Mean (pg/mL)	33.2	1746
Intra-assay SD	1.19	124
Intra-assay CV (%)	3.6	7.1
Inter-Mean (pg/mL)	32.3	1744
Inter-assay SD	2.05	136
Inter-assay CV (%)	6.3	7.8

#### Correlation

This assay has been correlated to the Quantikine® ELISA Kit with an R<sup>2</sup> value greater than 0.9.

#### Recovery

Recovery was not necessary as MCP-1 shows natural linearity data from a neat sample to a 1:16 dilution.



Simple Plex Assay: Human CCL2/MCP-1, 2nd Generation

specification sheet

#### Linearity

Samples spiked with high concentrations of human MCP-1 were serially diluted with Sample Diluent to produce samples within the dynamic range of the assay.

DILUTION	PARAMETER	CCS (n=4)	SERUM (n=4)	EDTA PLASMA (n=4)	HEPARIN PLASMA (n=4)
1:2	Avg % of Expected	103	94	95	100
	Range (%)	95-117	92-96	78-111	92-105
1:4	Avg % of Expected	97	99	101	100
	Range (%)	85-116	96-104	96-106	95-105
1:8	Avg % of Expected	91	103	105	107
	Range (%)	78-107	100-106	101-111	99-111
1:16	Avg % of Expected	88	107	111	107
	Range (%)	74-102	103-110	101-118	94-115

#### Specificity

This assay recognizes natural and recombinant human MCP-1. The factors listed were prepared at 34 ng/mL in Sample Diluent and assayed for cross-reactivity. Preparations of the following factors at 34 ng/mL in a rhMCP-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

#### Recombinant human:

- GRO $\alpha$
- GRO $\beta$
- GRO $\gamma$
- IL-8
- MCP-2
- MCP-3
- MCP-4
- MIP-1 $\alpha$
- MIP-1 $\beta$
- RANTES

#### Sample Collection and Storage

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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

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

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

*Note: Grossly hemolyzed or icteric samples are not suitable for use with this assay.*

#### Sample Preparation

Cell culture supernatant, serum, and plasma samples require a minimum 2-fold dilution with sample diluent. A suggested 2-fold dilution can be achieved by adding 35  $\mu\text{L}$  of sample to 35  $\mu\text{L}$  of Sample Diluent. Samples above the ULOQ require further dilution.

R&D SYSTEMS  
a biotechne brand



Toll-free: (888) 607-9692  
Tel: (408) 510-5500  
Fax: (408) 510-5599  
info@proteinsimple.com  
proteinsimple.com

© 2017 ProteinSimple, ELISA, Simple Plex and the ProteinSimple logo are trademarks and/or registered trademarks of ProteinSimple.

D16-1160-001 / Rev. A

