牛白介素 8(IL8)ELISA 试剂盒

货 号:	JYM0035Bo-T	检测范围:	31.25pg/mL-2000pg/mL
种 属:	牛	保存温度:	2-8℃
规 格:	96T/48T	有 效 期:	6个月
用 途:	用于体外定量检测细胞、血清、血浆以及其他样本中的牛IL8。		

※ 实验原理

本试剂盒采用双抗体夹心法 ELISA 技术：将捕获抗体包被于酶标板上，捕获样品及标准品中的待测物 IL8，洗涤后，再加入生物素标记的检测抗体进行孵育后清洗，形成“捕获抗体-抗原-检测抗体”免疫复合物，随后加入链霉亲和素偶联的辣根过氧化物酶进行孵育，待孵育结束后清洗，接着加入 TMB 显色后，若样本中有待测物则显蓝色，加入终止液停止反应。检测过程中游离的成分均被洗去，用酶标仪在 450 nm 处测 OD 值，颜色的深浅和样品中的待测物的含量呈正比，通过绘制标准曲线计算出样本中 IL8 的浓度。

※ 注意事项

1. **本试剂盒仅供教研使用，不可作为体外诊断依据；**
2. 预包被酶标板拆封后，未使用的板条请装入自封袋密封。为降低不同时间检测板内变异系数，间隔 48 小时内检测可 2- 8℃保存，如果间隔时间较长，请-20℃保存，并在下次检测时重新绘制标准曲线；
3. 浓缩洗涤液、浓缩生物素化抗体、浓缩 HRP 酶结合物请按要求稀释后使用，按需配置，现配现用；
4. 显色液 A 和显色液 B 建议先用一次性试管混匀后再添加到酶标板中，现配现用。配好的显色液应保持无色，直到添加到板中；
5. **此试剂盒提供的终止液为稀硫酸溶液，具有一定腐蚀性，应谨慎操作。如果不小心沾到皮肤上，请立即擦去，并用清水冲洗；**
6. **请严格按照说明书进行操作；如有疑问，请一定与技术工程师确认后再进行操作，避免样本和时间上的浪费；**
7. 不同批号试剂不建议混用，请勿使用其他品牌来源的试剂；
8. 封板膜、吸水纸、加样过程中所用的 EP 管和吸头为一次性使用，严禁混用。

※ 试剂盒组成

<table>
<tr><th>中文名称</th><th>96T 配置</th><th>48T 配置</th><th>保存条件</th></tr>
<tr><td>预包被酶标板</td><td>8 孔×12 条</td><td>8 孔×6 条</td><td rowspan="9">2-8℃</td></tr>
<tr><td>标准品</td><td>1 支×200μL</td><td>1 支×100μL</td></tr>
<tr><td>100×生物素化抗体</td><td>1 支×100μL</td><td>1 支×50μL</td></tr>
<tr><td>100×SA-HRP</td><td>1 支×100μL</td><td>1 支×50μL</td></tr>
<tr><td>20×浓缩稀释液</td><td>1 瓶×25mL</td><td>1 瓶×15mL</td></tr>
<tr><td>显色液 A</td><td>1 瓶×6mL</td><td>1 瓶×3mL</td></tr>
<tr><td>显色液 B</td><td>1 瓶×6mL</td><td>1 瓶×3mL</td></tr>
<tr><td>终止液</td><td>1 瓶×6mL</td><td>1 瓶×3mL</td></tr>
<tr><td>20×浓缩洗涤液</td><td>1 瓶×25mL</td><td>1 瓶×15mL</td></tr>
<tr><td>封板膜</td><td>4 张</td><td>4 张</td><td rowspan="2">RT</td></tr>
<tr><td>产品说明书</td><td>1 份</td><td>1 份</td></tr>
</table>

※ 需要而未提供的物品：

仪器设备	**其它**
含 450nm 检测波长的酶标仪 各种量程移液器 可提供 37°C 环境的恒温箱或培养箱	吸水纸或者抽纸 蒸馏水或去离子水 各种规格吸头和 EP 管

※ 样本处理及要求：

1、 **血清：**将收集于血清分离管的全血标本在室温静置 30min 以上，不超过 2 小时，然后 2-8℃，2500-3500×g 离心 20min，仔细收集上清；

2、 **血浆：**应根据标本的要求选择 EDTA 或柠檬酸钠作为抗凝剂，样品采集后 30min 内在 2-8℃，3000×g 离心 15min，仔细收集上清。保存过程中如有沉淀形成，应再次离心；

3、 **尿液：**用无菌管收集，2-8℃，2500-3500×g 离心 10min，仔细收集上清。保存过程中如有沉淀形成，应再次离心。胸腹水、脑脊液参照实行；

4、 **细胞培养上清：**收集液体后于 2-8℃，2500-3500×g 离心 20min，除去杂质及细胞

碎片，取上清检测。

5、 **细胞裂解液：**贴壁细胞用预冷的 PBS(0.01M,pH=7.4)轻轻清洗，然后用胰蛋白酶消化，2-8℃，1000×g 离心 5min 后收集细胞；悬浮细胞可直接离心收集。收集的细胞用冷的 PBS 洗涤 3 次。每 1×10^6 个细胞中加入 150-200μL PBS 重悬，并通过反复冻融或超声使细胞破碎(推荐在 PBS 中加入蛋白酶抑制剂；若含量很低可减少 PBS 的体积)。将提取液于 2-8℃，10000×g 离心 10min，取上清检测；

6、 **组织样本：**用预冷的 PBS(0.01M,pH=7.4)冲洗组织，去除残留血液，称重后将组织剪碎。将剪碎的组织与对应体积的 PBS(一般按 1:9 的重量体积比，比如 1 g 的组织样品对应 9 mL 的 PBS，具体体积可根据实验需要适当调整，并做好记录。推荐在 PBS 中加入蛋白酶抑制剂)加入匀浆器中，在冰上充分研磨。为了进一步裂解组织细胞，可以对匀浆液进行反复冻融或超声破碎。最后将匀浆液于 2- 8℃，10000×g 离心 5-10min，取上清检测；

7、 **其它生物标本：**2- 8℃，2500-3500×g 离心 20min，仔细收集上清。

样品外观：样品应清澈透明，悬浮物应离心去除。

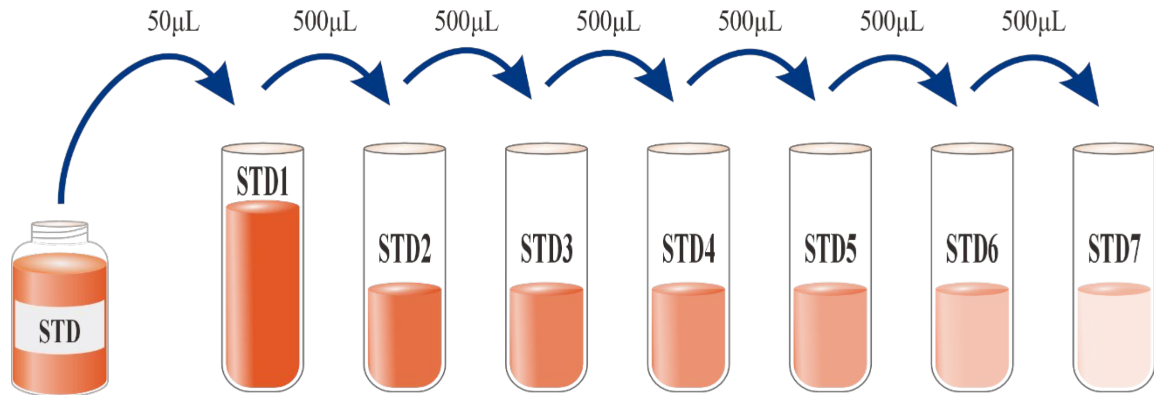
样品保存：样品收集后尽快检测。若不能及时检测，请按一次使用量分装，冻存于-20℃(1 个月内检测)，或-80℃(6 个月内检测)，避免反复冻融。

※ 试剂准备工作

使用前将所有试剂置于室温平衡 30 分钟左右。

洗涤液/稀释液配置：如果洗涤液/稀释液（20×）有晶体析出，需在 37℃下加热至晶体全部溶解。用蒸馏水 1:20 稀释（例如：1mL 浓缩洗涤液加入 19mL 的蒸馏水）。

标准品配置：试剂盒中取出标准品，准备 7 个试管，先从 40 ng/mL 标准品（200uL）按需吸取一定量用 1×稀释液稀释至 2000pg/mL（例：50uL 的标准品母液+950uL 的 1×稀释液，制备得到 1000μL 的 2000pg/mL 浓度标准品），随后在 6 个试管中分别加入 500μL 的 1×稀释液，在这 6 个单独的试管中将 2000pg/mL 标准品依次 2 倍倍比稀释至 6 个梯度，共配制 7 个浓度的标准品，依次为：2000pg/mL、1000pg/mL、500pg/mL、250pg/mL、125pg/mL、62.5pg/mL、31.25pg/mL，从最高浓度标准品溶液中吸取 500μL 标准品到下一个试管中，轻轻吹打混匀，以此类推进行标准品的倍比稀释（如图所示），1×稀释液用作零浓度标准品(0pg/mL)。



稀释后各管中标准品浓度如下(单位：pg/mL)

STD	STD1	STD2	STD3	STD4	STD4	STD6	STD7
40 ng/mL	2000	1000	500	250	125	62.5	31.25

抗体工作液配置：使用前 10 分钟，将 100×生物素化抗体于 1000×g 离心 1 分钟，随后用 1×稀释液将 100×生物素化抗体稀释成 1×生物素化抗体工作液，根据所需用量当日配置当日使用。

酶结合物工作液配置：使用前 10 分钟，将 100×SA-HRP 溶液于 1000×g 离心 1 分钟，随后用 1×稀释液将 100×SA-HRP 稀释成 1×SA-HRP 工作液，根据所需用量当日配置当日使用。

备注：如待测样本中 IL8 浓度高于标准品最高值，请根据实际情况选择适当的稀释倍数。

※ 实验步骤

所有标准品、样品建议复孔检测

1. 酶标板准备：确定实验所需要的孔数，取下其它不使用的板条放回装有干燥剂的密封袋；
2. 样本孵育：分别加入 100μL 不同浓度的标准品以及预处理过的待测样品（建议样本用通用稀释液最少稀释 1 倍上样，目的是减少基质效应，**空白孔加 1×稀释液**），盖上封板膜，37℃避光反应 1 h。孵育结束后，每孔加入 300μL 1×洗涤缓冲液，轻轻晃动 30 秒，甩干并在吸水纸上拍干，以这种方式清洗 3 次；
3. 抗体孵育：每孔加入 100μL 生物素化抗体工作液，轻轻混匀，盖上封板膜，37℃避光反应 1h。孵育结束后，重复步骤 2 中的清洗方式清洗 4 次；
4. 酶标孵育：每孔加入 100μL 1×SA-HRP 工作液，盖上封板胶纸，37℃避光反应 30min，重复步骤 2 中的清洗方式清洗 4 次，**在新的吸水纸上拍干**；
5. 底物显色：将显色液 A 和显色液 B 按照 1：1 比例混匀配成底物显色液（按需配置，

确认此时应为无色透明)，每孔加入 100μL 配好的显色液，盖上封板膜，37℃避光反应 15 分钟；

6. 终止反应：待显色反应结束后，每孔加入 50μL 终止液，轻轻晃动酶标板混匀，5 分钟内用预热完成的酶标仪在 450nm 处测吸光值。

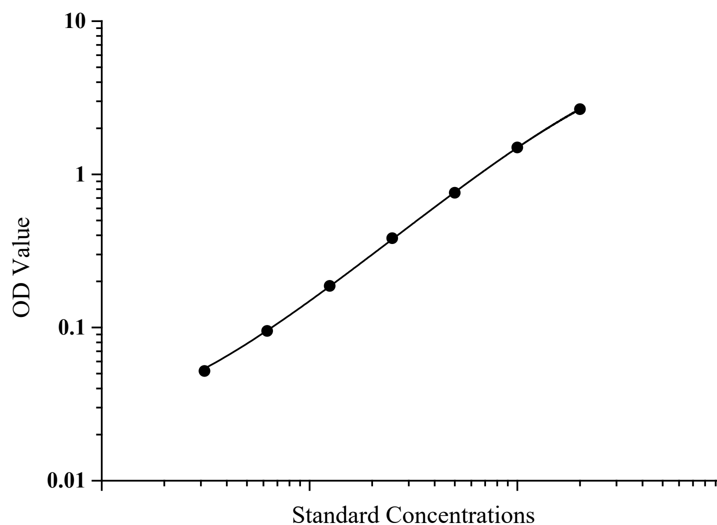
※ 结果的计算

计算标准品和样本复孔的平均 OD 值并减去空白孔的 OD 值作为校正值。以浓度为横坐标，OD 值为纵坐标，在坐标纸上绘出四参数逻辑函数的标准曲线(作图时去掉空白组的值)。或者使用能够生成四参数逻辑（4-P）曲线拟合的计算机软件来创建标准曲线。若样品 OD 值高于标准曲线上限，应适当稀释后重测并在计算样本浓度时乘以相应的稀释倍数。

※ 示例数据

以下数据和曲线仅供参考，实验者需根据自己的实验数据建立标准曲线。

标准品浓度 (pg/mL)	2000	1000	500	250	125	62.5	31.25	0
OD 值	2.705	1.531	0.793	0.42	0.229	0.133	0.086	0.038
校正 OD 值	2.667	1.493	0.755	0.382	0.191	0.095	0.048	0



本图所示标准曲线仅供示例，结果计算应以同次试验标准品所绘标准曲线为准计算样本结果。

精密度

板内，板间变异系数均<10%。

板内精密度：在同一块板上重复检测三个已知浓度的样品 20 次，计算浓度的变异系数（CV）。

板间精密度：在三块板子上对三个已知浓度的样品分别进行 20 次重复检测，计算浓度的变异系数（CV）。

	板内精密度			板间精密度		
样本	1	2	3	1	2	3
数量	20	20	20	20	20	20
平均值（pg/mL）	62.28	124.74	249.89	62.74	125.14	250.16
标准差	0.96	2.68	7.37	1.52	3.28	8.61
变异系数（%）	1.54	2.15	2.95	2.43	2.62	3.44

回收率

分别往不同样本中添加已知浓度的牛 IL8，做回收实验，得出回收率范围和平均回收率。

样本类型	范围（%）	平均回收率（%）
血清(n=8)	89-103	95
血浆(n=8)	95-101	95
细胞培养物(n=8)	98-116	108

灵敏度

经样本测试，本试剂盒的检测灵敏度为 15.62pg/mL。

线性关系

将高浓度牛IL8加入样本中，在标准曲线范围内分别稀释2倍，4倍，8倍，16倍做回收实验，得出回收率及平均回收率。

		血清（n=4）	细胞培养物(n=4)
1：2	回收率范围（%）	95-111	94-106
	平均回收率（%）	99	100
1：4	回收率范围（%）	99-115	96-111
	平均回收率（%）	103	104
1：8	回收率范围（%）	104-117	100-114
	平均回收率（%）	105	111
1：16	回收率范围（%）	106-121	105-122
	平均回收率（%）	112	119

特异性

该试剂盒测定可识别重组牛 IL8。

其他相关蛋白在稀释缓冲液中制备为 50ng/mL，并测定交叉反应性。没有观察到明显的交叉反应。

Bovine Interleukin 8 (IL8) ELISA Kit Manual

Cat. No.:	JYM0035Bo-T	**Detection Range:**	31.25pg/mL-2000pg/mL
Species:	Bovine	**Storage Temp.:**	2-8℃
Format:	96T/48T	**Shelf Life:**	6 months
Intended Use: For quantitative in vitro detection of Bovine IL8 in cultured cells, serum, plasma and other samples.			

Assay Principle

This kit employs double antibody sandwich ELISA technology: **Capture Antibody** is coated on the microplate to capture IL8 from samples and standards. After washing, biotin-labeled **Detection Antibody** is added and incubated, followed by washing to form a "**Capture Antibody-Antigen-Detection Antibody**" immune-complex. Subsequently, Streptavidin-Horseradish Peroxidase (**SA-HRP**) is added and incubated. After incubation and washing, **TMB Substrate** is added for color development. If the target analyte is present in the sample, a blue color develops. **Stop Solution** is then added to terminate the reaction. During the assay, unbound components are washed away. The **Optical Density** (OD) is measured at 450 nm using a microplate reader. The intensity of the color is proportional to the IL8 concentration in the sample, and the concentration is calculated by plotting a standard curve.

Precautions

1. **This kit is for research use only. Not for use in diagnostic procedures.**
2. After opening the sealed bag containing the pre-coated strips, any unused strips should be immediately resealed in a ziplock bag with desiccant. To minimize inter-assay variation, strips can be stored at 2-8°C if the next assay is within 48 hours. For longer intervals, store at -20°C and re-run the standard curve in the next assay.
3. The Concentrated Wash Buffer, Concentrated Biotinylated Antibody, and Concentrated HRP Conjugate must be diluted as instructed before use. Prepare these solutions as needed on the day of use.
4. It is recommended to mix Substrate Solution A and Substrate Solution B in a disposable tube first before adding to the wells. Prepare the mixed substrate solution immediately

before use. The prepared substrate solution should remain colorless until added to the plate.

5. **The Stop Solution provided in this kit is a dilute sulfuric acid solution, which is corrosive. Handle with care. If contact with skin occurs, wipe off immediately and rinse thoroughly with water.**
6. **Operate strictly according to the instructions. If in doubt, please confirm with technical support before proceeding to avoid waste of samples and time.**
7. Do not mix reagents from different lot numbers. Do not use reagents from other sources or manufacturers.
8. Seal plates, absorbent paper, EP tubes, and pipette tips used during sample addition are for single use only. Do not reuse.

Kit Components

<table>
<tr><th>Component</th><th>96T Config</th><th>48T Config</th><th>Storage Condition</th></tr>
<tr><td>Pre-coated Plate</td><td>8 wells × 12 strips</td><td>8 wells × 6 strips</td><td rowspan="9">2-8°C</td></tr>
<tr><td>Standard</td><td>1 vial × 200μL</td><td>1 vial × 100μL</td></tr>
<tr><td>100× Biotinylated Ab</td><td>1 vial × 100μL</td><td>1 vial × 50μL</td></tr>
<tr><td>100× SA-HRP</td><td>1 vial × 100μL</td><td>1 vial × 50μL</td></tr>
<tr><td>20× Diluent</td><td>1 bottle × 25mL</td><td>1 bottle × 15mL</td></tr>
<tr><td>Substrate A</td><td>1 bottle × 6mL</td><td>1 bottle × 3mL</td></tr>
<tr><td>Substrate B</td><td>1 bottle × 6mL</td><td>1 bottle × 3mL</td></tr>
<tr><td>Stop Solution</td><td>1 bottle × 6mL</td><td>1 bottle × 3mL</td></tr>
<tr><td>20× Wash Buffer</td><td>1 bottle × 25mL</td><td>1 bottle × 15mL</td></tr>
<tr><td>Plate Sealer</td><td>4 sheets</td><td>4 sheets</td><td rowspan="2">RT</td></tr>
<tr><td>Instruction</td><td>1 copy</td><td>1 copy</td></tr>
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Materials Required but Not Provided:

Equipment & Instruments	**Other Materials**
Microplate reader with 450 nm filter	Absorbent paper or lab wipes
Pipettes of various volumes	Distilled or deionized water
Incubator capable of maintaining 37°C	Various pipette tips and EP tubes

Sample Collection and Handling:

1. **Serum:** Collect whole blood into serum separation tubes. Allow clotting at room temperature for 30 minutes to 2 hours. Centrifuge at 2-8°C, 2500-3500 × g for 20 minutes. Carefully collect the supernatant.
2. **Plasma:** Use EDTA or citrate as anticoagulant as required. Centrifuge samples within 30 minutes of collection at 2-8°C, 3000 × g for 15 minutes. Carefully collect the supernatant. If precipitation occurs during storage, re-centrifuge.
3. **Urine:** Collect using sterile tubes. Centrifuge at 2-8°C, 2500-3500 × g for 10 minutes. Carefully collect the supernatant. If precipitation occurs during storage, re-centrifuge. Handle pleural fluid, ascitic fluid, and cerebrospinal fluid similarly.
4. **Cell Culture Supernate:** Collect the fluid and centrifuge at 2-8°C, 2500-3500 × g for 20 minutes to remove impurities and cell debris. Collect the supernatant for assay.
5. **Cell Lysate:** Wash adherent cells gently with ice-cold PBS (0.01M, pH=7.4), then digest with trypsin. Collect cells by centrifugation at 2-8°C, 1000 × g for 5 minutes. Suspension cells can be collected directly by centrifugation. Wash collected cells 3 times with cold PBS. Resuspend 1×10^6 cells in 150-200μL PBS. Lyse cells by repeated freeze-thaw cycles or ultrasonication (it is recommended to add protease inhibitors to PBS; if the target concentration is expected to be low, reduce the PBS volume). Centrifuge the lysate at 2-8°C, 10000 × g for 10 minutes. Collect the supernatant for assay.
6. **Tissue Samples:** Rinse the tissue with ice-cold PBS (0.01M, pH=7.4) to remove residual blood. Weigh and mince the tissue. Add the minced tissue to an appropriate volume of PBS (generally a 1:9 weight/volume ratio, e.g., 1g tissue to 9mL PBS; the volume can be adjusted according to experimental needs and should be recorded. It is recommended to add protease inhibitors to PBS) in a homogenizer. Grind thoroughly on ice. To further lyse cells, subject the homogenate to repeated freeze-thaw cycles or ultrasonication. Finally, centrifuge the homogenate at 2-8°C, 10000 × g for 5-10 minutes. Collect the supernatant for assay.
7. **Other Biological Samples:** Centrifuge at 2-8°C, 2500-3500 × g for 20 minutes. Carefully collect the supernatant.

Sample Appearance: Samples should be clear and transparent. Suspended matter should be removed by centrifugation.

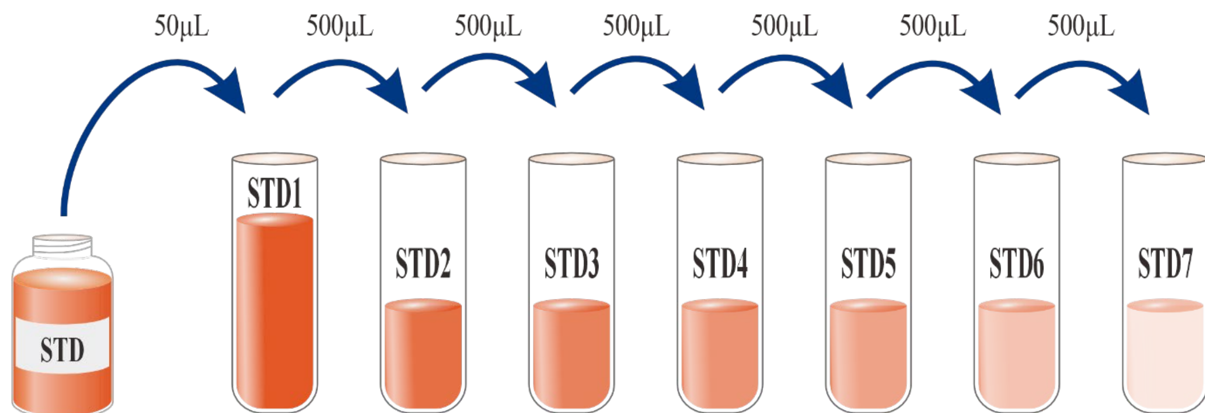
Sample Storage: Assay samples as soon as possible after collection. If testing cannot be performed immediately, aliquot the samples and store at -20°C (test within 1 month) or -80°C (test within 6 months). Avoid repeated freeze-thaw cycles.

Reagent Preparation

Allow all reagents to equilibrate to room temperature for about 30 minutes before use.

Wash Buffer/Diluent Working Solution Preparation: If crystals have formed in the concentrated (20×) Wash Buffer/Diluent, warm it at 37°C until all crystals are completely dissolved. Dilute 1:20 with distilled water (e.g., add 1 mL of concentrated Wash Buffer to 19 mL of distilled water).

Standard Preparation: Take out the standard from the kit. Prepare 7 tubes. First, dilute the 40 ng/mL standard (200uL) as needed with 1× Diluent to a concentration of 2000pg/mL (e.g., 50uL of standard stock + 950uL of 1× Diluent to prepare 1000μL of 2000pg/mL standard). Then, add 500μL of 1× Diluent to each of 6 separate tubes. Perform a serial 2-fold dilution of the 2000pg/mL standard in these 6 tubes to create 6 gradients, resulting in a total of 7 standard concentrations: 2000pg/mL, 1000pg/mL, 500pg/mL, 250pg/mL, 125pg/mL, 62.5pg/mL, and 31.25pg/mL. Transfer 500μL from the highest concentration standard to the next tube, mix gently by pipetting, and so on for the serial dilution. Use 1× Diluent as the zero standard (0pg/mL).



Concentrations after dilution are as follows (Unit: pg/mL)

STD (Stock)	STD1	STD2	STD3	STD4	STD5	STD6	STD7
40 ng/mL	2000	1000	500	250	125	62.5	31.25

Biotinylated Ab Working Solution Preparation: 10 minutes before use, centrifuge the 100× Biotinylated Antibody at 1000 × g for 1 minute. Then dilute the 100× Biotinylated Antibody with 1× Diluent to prepare the 1× Biotinylated Antibody working solution. Prepare this working solution on the day of use according to the required volume.

SA-HRP Working Solution Preparation: 10 minutes before use, centrifuge the 100× SA-HRP solution at 1000 × g for 1 minute. Then dilute the 100× SA-HRP with 1× Diluent to prepare the

1× SA-HRP working solution. Prepare this working solution on the day of use according to the required volume.

Note: If the IL8 concentration in the sample is higher than the highest standard point, dilute the sample with an appropriate dilution factor as needed and re-assay. Multiply the result by the dilution factor.

Assay Procedure

All standards and samples are recommended to be assayed in duplicate.

1. **Microplate Preparation:** Determine the number of strips required for the assay. Remove other unused strips and immediately return them to the sealed bag containing desiccant.
2. **Sample Incubation:** Add 100μL of different concentration standards and pre-treated samples to the appropriate wells (it is recommended to dilute samples at least 1-fold with the general diluent before adding to reduce matrix effects,**and add 1× Diluent buffer to the blank well.**). Cover with the plate sealer. Incubate at 37°C protected from light for 1 hour. After incubation, aspirate or decant the liquid from each well. Add 300μL of 1× Wash Buffer to each well, gently swirl for 30 seconds, then discard the contents. Blot the plate dry by tapping it firmly onto absorbent paper. Repeat this wash step 3 times.
3. **Antibody Incubation:** Add 100μL of Biotinylated Antibody working solution to each well. Mix gently. Cover with a new plate sealer. Incubate at 37°C protected from light for 1 hour. After incubation, repeat the wash procedure as in step 2, 4 times.
4. **Enzyme Conjugate Incubation:** Add 100μL of 1× SA-HRP working solution to each well. Cover with a new plate sealer. Incubate at 37°C protected from light for 30 minutes. After incubation, repeat the wash procedure as in step 2, 4 times. **Blot the plate dry on fresh absorbent paper.**
5. **Substrate Development:** Mix Substrate Solution A and Substrate Solution B in a 1:1 ratio to prepare the substrate working solution (prepare as needed; it should be colorless and transparent). Add 100μL of the prepared substrate working solution to each well. Cover with a new plate sealer. Incubate at 37°C protected from light for 15 minutes.
6. **Stop Reaction:** After the color development, add 50μL of Stop Solution to each well. Gently tap the plate to mix. Measure the Optical Density (OD) at 450 nm using a pre-warmed microplate reader within 5 minutes.

Calculation of Results

Calculate the average OD value for each standard and sample duplicate, then subtract the average OD value of the zero standard (blank) well to obtain the corrected OD value. Plot the corrected OD values (y-axis) against the corresponding standard concentrations (x-axis) on graph paper using a four-parameter logistic (4-PL) curve fit (omit the zero standard value when plotting).

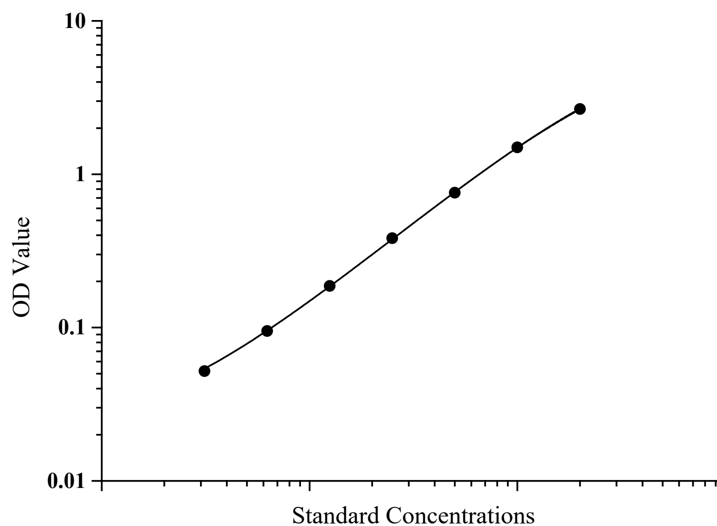
Alternatively, use computer software capable of generating a four-parameter logistic (4-PL) curve fit to create the standard curve.

If the OD value of a sample is higher than the highest standard point, the sample should be appropriately diluted and re-assayed. Multiply the concentration obtained from the standard curve by the dilution factor.

Typical Data

The following data and curve are for reference only. The experimenter must establish a standard curve based on their own experimental data.

Standard Conc. (pg/mL)	2000	1000	500	250	125	62.5	31.25	0
OD Value	2.705	1.531	0.793	0.42	0.229	0.133	0.086	0.038
Corrected OD Value	2.667	1.493	0.755	0.382	0.191	0.095	0.048	0



The standard curve shown in this figure is for example purposes only. Use the standard curve generated from the standards in the same assay to calculate sample results.

Precision

Intra-assay and inter-assay Coefficients of Variation (CV) are both <10%.

- **Intra-assay Precision:** Three known concentration samples were assayed 20 times on one plate. The CV of the concentrations was calculated.
- **Inter-assay Precision:** Three known concentration samples were assayed in 20 replicates across three different plates. The CV of the concentrations was calculated.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	62.28	124.74	249.89	62.74	125.14	250.16
Standard Deviation	0.96	2.68	7.37	1.52	3.28	8.61
CV (%)	1.54	2.15	2.95	2.43	2.62	3.44

Recovery

Recovery was tested by spiking known concentrations of Bovine IL8 into different sample matrices. The recovery range and average recovery are shown below.

Sample Type	Range (%)	Average Recovery (%)
Serum (n=8)	89-103	95
Plasma (n=8)	95-101	99
Cultured Cells (n=8)	98-116	108

Sensitivity

The minimum detectable concentration (sensitivity) of Bovine IL8, determined by testing samples, is 15.62pg/mL.

Linearity

High concentration Bovine IL8 was spiked into samples and then serially diluted 2-fold, 4-fold, 8-fold, and 16-fold within the range of the standard curve to assess linearity. Recovery rates and average recovery are shown below.

Dilution		Serum (n=4)	Cultured Cells (n=4)
1:2	Recovery Range (%)	95-111	94-106
	Avg. Recovery (%)	99	100
1:4	Recovery Range (%)	99-115	96-111
	Avg. Recovery (%)	103	104
1:8	Recovery Range (%)	104-117	100-114
	Avg. Recovery (%)	105	111
1:16	Recovery Range (%)	106-121	105-122
	Avg. Recovery (%)	112	119

Specificity

This kit specifically detects recombinant Bovine IL8. Other related proteins were prepared at 50 ng/mL in the dilution buffer and tested for cross-reactivity. No significant cross-reactivity was observed.