HMGB1 Detection ELISA kit

高迁移率族蛋白 B1(HMGB1)酶联免疫吸附测定试剂盒

Catalog # 6010

（产品货号 6010）

For Research Use Only - Not Human or Therapeutic Use

（仅供科研使用，不用临床诊断治疗）

PRODUCT SPECIFICATIONS 产品规格

DESCRIPTION 产品简介:	ELISA kit to quantify HMGB1 检测生物样本中高迁移率族蛋白 B1 水平
FORMAT 试剂盒规格:	96-well ELISA Plate with removeable strips 96 孔板
ASSAY TYPE 检测试剂盒类型:	Sandwich ELISA 双抗体夹心酶联免疫吸附检测技术
ASSAY TIME 手工操作时间:	2 hours* 2 小时*
STANDARD RANGE 检测范围:	50 ng/ml to 0.8 ng/ml
NUMBER OF SAMPLES 样本量:	Up to 40 (duplicate) samples/plate 可检测 40 个样本，每个样本可重复两次
SAMPLE TYPES 样本类型:	Cell culture, Serum, and Plasma 细胞培养上清，血清和血浆
RECOMMENDED SAMPLE DILUTIONS: 建议样本稀释比例	1:1 (at least)
CHROMOGEN 显色剂:	TMB (read at 450 nm) （吸光度 450nm）
STORAGE 保存条件:	-20°C
VALIDATION DATA 验证数据:	Human Serum人血清: Intra-Assay板内变异系数 (2.7-3.6%)/Inter-Assay板间变异系数 (1.3-5.1%)/Recovery回收率 (101+/-25.1%) Mouse Serum小鼠血清: Intra-Assay板内变异系数(1.4-8.1%)/Inter-Assay板间变异系数 (0.9-4.5%)/Recovery回收率 (104+/8.3%)
NOTES 注意事项:	*This kit has an overnight incubation step *含过夜孵育步骤

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16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

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INTRODUCTION 产品介绍

HMGB1 (high mobility group box 1) (1) was recently rediscovered as a late lethal mediator of endotoxin (2) and is currently considered a pro-inflammatory cytokine that plays crucial roles in a variety of acute and chronic inflammatory diseases. HMGB1 contains 216 amino acids (3) and maintains 99% of its sequence identity among mice (4), rats (5), bovines (6), and humans (7). HMGB1 consists of three structural domains (8), termed “A box (9-85)”, “B box (88-162)”, and a negatively charged carboxyl terminus (186-216). Moreover, it has been previously shown that the B box recapitulates the pro-inflammatory activity whereas the A box acts as an antagonist of HMGB1 (9,10).

近些年研究显示高迁移率族蛋白1(HMGB1)（1）是内毒素血症和脓毒症的晚期炎性介质（2）。HMGB1作为促炎因子在调节急性和慢性炎症疾病中起重要作用。HMGB1由216个氨基酸组成(3)，它的结构在不同物种的真核细胞中高度保守，其中小鼠(4)，大鼠(5)，牛(6)和人（7）的氨基酸序列具有99%同源性。HMGB1有三个独特的结构域组成（8），分别为A盒（9-85）， B盒（88-162）,和残基C末端（186-216）。其中B盒具有促炎作用， A盒能竞争性抑制HMGB1，成为HMGB1的拮抗剂（9，10）。

Several lines of evidence highlight the significance of HMGB1 in the immune inflammatory response. For example, it has been shown that HMGB1 is actively released by a variety of cells such as macrophages when stimulated by lipopolysaccharides (LPS), TNF-α, and IL-1 (2), and is passively released by injured or necrotic cells associated with collapsing cell structures. In fact, patients who died from septic shock had higher serum HMGB1 levels than surviving sepsis patients (11). Similarly, high serum HMGB1 levels are observed in sepsis animal models and in collagen-induced arthritis animal models (12). With regard to the function of the protein itself, HMGB1 has also been shown to stimulate the release of TNF-α and IL-1 (13,14), as well as bind LPS and synergistically increase peripheral blood mononuclear cell IL-6 production (15). Together, these observations demonstrate that HMGB1 plays important roles in the inflammatory cascade.

多项研究表明HMGB1在炎性反应中起到重要作用。例如，在脂多糖（LPS），肿瘤坏死因子a（TNF-a）和白介素-1（IL-1）的刺激下， 各种细胞例如巨噬细胞会主动释放HMGB1， 而受损或坏死细胞则会被动释放HMGB1。事实上，死于感染性休克的患者血清中的HMGB1水平高于存活的败血症患者 （11）。在败血症动物模型和胶原蛋白诱导的关节炎动物模型中，血清HMGB1水平也显著升高。关于该蛋白本身的功能，研究表明HMGB1能够刺激TNF-a和IL-1的释放 （13，14），并能够结合LPS并协同增加外周血单核细胞白介素-6 （IL-6）的产生（15）。总而言之，这些观察结果表明HMGB1在炎症级联反应中发挥着重要作用。

Chondrex, Inc. provides an HMGB1 Detection ELISA kit (Cat # 6010) to determine HMGB1 levels in cell culture media and sera. This kit contains enough reagents to measure 40 samples in duplicate together with standards.

Chondrex公司提供HMGB1检测试剂盒（#6010）。该试剂盒用于体外定量检测细胞培养液和血清中的HMGB1浓度。这个试剂盒可以用于对40个样本进行重复检测，并附带标准品。

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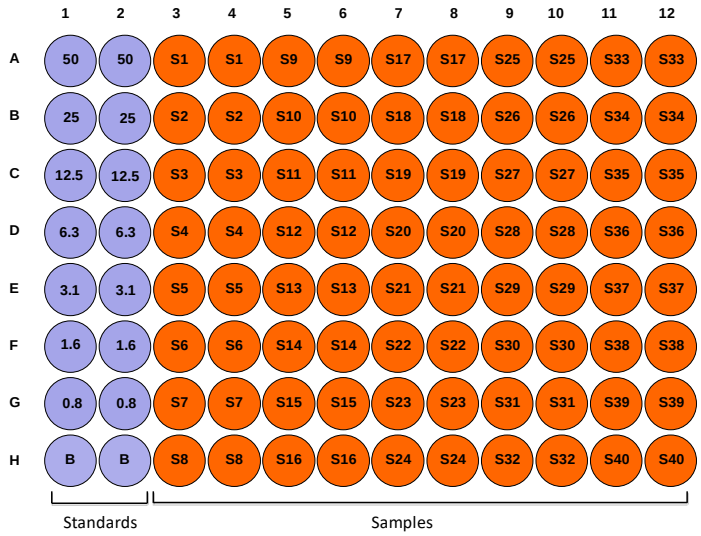
KIT COMPONENTS 试剂盒组分及保存

Item 试剂	Quantity 数量	Amount 规格	Storage 保存条件
HMGB1 Standard (60101) HMGB1 标准品（60101）	2 vials 2 管	50 µl/vial 50 µl/管	-20°C
Capture Antibody (Anti-HMGB1 Monoclonal Antibody) (60102) 捕获抗体（抗 HMGB1 单克隆抗体）（60102）	1 vial 1 管	100 µl/vial 100 µl/管	-20°C
Detection Antibody (Anti-HMGB1 Monoclonal Antibody) (60103) 检测抗体（抗 HMGB1 单克隆抗体）（60103）	1 vial 1 管	Lyophilized 冻干的	-20°C
Solution A - Coating Buffer (9052) 试剂 A-捕获抗体稀释液（9052）	1 bottle 1 瓶	10 ml	-20°C
Solution B - Sample/Standard Dilution Buffer (601010) 试剂 B-样本/标准品稀释液（601010）	1 bottle 1 瓶	20 ml	-20°C
Solution C - Detection Antibody Dilution Buffer (60106) 试剂 C-检测抗体稀释液（60106）	1 bottle 1 瓶	10 ml	-20°C
Solution D - Streptavidin Peroxidase Dilution Buffer (9055) 试剂 D-辣根过氧化物酶标记链霉亲和素稀释液（9055）	1 bottle 1 瓶	20 ml	-20°C
Streptavidin Peroxidase (9029) 辣根过氧化物酶标记链霉亲和素（9029）	2 vials 2 管	50 µl	-20°C
TMB Solution (contains DMSO) (90023) 显色剂（含 DMSO）(90023)	2 vials 2 管	0.2 ml	-20°C
Chromogen Dilution Buffer (90022) 显色剂稀释液（90022）	1 bottle 1 瓶	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid (9016) 终止液	1 bottle 1 瓶	10 ml	-20°C
Wash Buffer, 20X (9005) 浓缩洗涤液 20X（9005）	1 bottle 1 瓶	50 ml	-20°C
ELISA Plate ELISA 酶标板	1 each 一块	96-well (8-well strips x 12) 96 孔（8 孔 X 12 条）	-20°C

PLATE LAYOUT 加样布局



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ASSAY OUTLINE 实验操作步骤一览表

Add 100 µl of diluted Capture Antibody Solution to wells.
每孔加入100 µl 1X捕获抗体

Incubate plate at 4°C overnight.
4 ° C 孵育过夜

Wash plates. 洗板

Add 50 µl of diluted standards and samples to wells.
每孔加入50 µl 1X 标准品或样本

+

Add 50 µl of diluted Detection Antibody Solution to wells.
每孔加入50 µl 1X 检测抗体

Mix plate with a plate mixer or *pipet individual wells.
Incubate at 37°C for 1 hour.
Transfer plate to 4°C overnight.
充分混匀，37 °C 孵育 1 小时，然后4 °C孵育过夜。

Wash plates. 洗板

Add 100 µl of diluted Streptavidin Peroxidase Solution to wells.
每孔加入100 µl 1X 辣根过氧化物酶标记亲和素

Incubate plate at room temperature for 30 minutes.
室温孵育30分钟

Wash plates. 洗板

Add 100 µl of diluted TMB solution to wells.
每孔加入100 µl 1X TMB显色液

Incubate plate at room temperature for 30 minutes.
室温孵育30分钟

Add 50 µl of Stop Solution to wells.
每孔加入50 µl 终止液

Read plate at 450 nm/630 nm.
用酶标仪在波长450nm/630nm处读取吸光度值

* Use one tip per sample or standards. Do not cross-contaminate samples or standards by re-using pipet tips. A multi-channel pipet is recommended.为避免交叉污染，不要重复使用吸头，建议用多通道移液器加样。

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NOTES BEFORE USING ASSAY 注意事项

NOTE 1: It is recommended that the standard and samples be run in duplicate.

1. 建议ELISA试验中标准品和样本都做复孔，尽量避免实验误差。

NOTE 2: Warm up all buffers to room temperature before use.

2. 请提前20-30分钟将试剂从冰箱中取出， 平衡至室温后使用。

NOTE 3: Crystals may form in Wash Buffer, 20X when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are completely dissolved.

3. 在4ºC条件下浓缩洗涤液可能会有结晶析出。如出现结晶，请放入37ºC温浴，直到结晶完全溶解后再配置1X洗涤液。

NOTE 4: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

4. 因为试剂盒提供了额外量的试剂，建议使用移液管测量确切体积的溶液。

NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

5. 在每个步骤之后盖上封板膜，以防板周围孔中的试剂挥发。

NOTE 6: For partial reagent use, please see the assay protocol's corresponding step for the appropriate dilution ratio. For example, if the protocol dilutes 50 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 25 µl of the stock solution in 5 ml of buffer. Partially used stock reagents may be kept in their original vials and stored at -20ºC for use in a future assay.

6. 配试剂时，请根据说明书中的指示配相应量的试剂。例如，12条8孔条，需用10 ml稀释液稀释50 µl的浓缩试剂，6条8孔条需要用5 ml稀释液稀释25 µl浓缩试剂。剩余试剂可继续保存在-20ºC。

NOTE 7: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

7. 本试剂盒含有动物成分，应按生物废弃物处理。

NOTE 8: This kit can be used to determine HMGB1 levels in sera and cell culture media samples. However, special concern should be considered for assaying HMGB1 in human serum because autoantibodies to HMGB1 are determined in 9-89% of sera from patients with autoimmune and inflammatory diseases (16-19). These reports indicate that human serum polyclonal antibodies to HMGB1 might mask the epitopes recognized by the capture and detection antibodies used in this kit, resulting in interference against the assay.

8. 本试剂盒用于检测血清和细胞培养液中HMGB1浓度。值得注意的是检测人类血清中的HMGB1时，只有在9-89%的自身免疫疾病的病人血清中可以检测到HMGB1（16-19）。研究表明人体血清中含有抗HMGB1多克隆抗体，可能会蒙蔽捕获或检测抗体识别的抗原表位，导致结果的干扰。

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ASSAY PROCEDURE 实验操作步骤

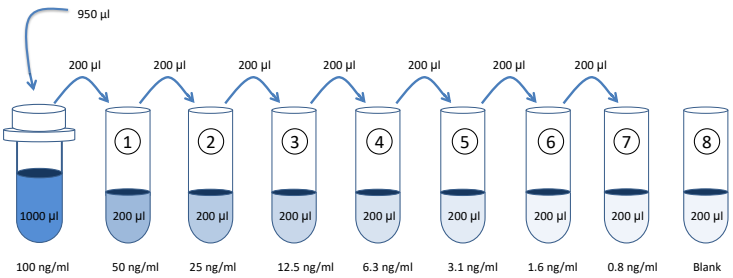
1. **Add Capture Antibody （加捕获抗体）**: Dilute 1 vial (100 µl) of Capture Antibody with 10 ml of Coating Buffer (Solution A). Add 100 µl of capture antibody solution to each well and incubate at 4°C overnight. If planning to use less, the remaining stock solution can be stored in its original vial at -20°C.

   用试剂 A 将 100X 捕获抗体稀释成 1X 的捕获抗体，例如，用 10 ml 试剂 A 稀释一管 100 µl 的捕获抗体， 然后在每孔中加入 100 µl 的 1X 捕获抗体，4°C 孵育过夜。稀释抗体前，请参考以下表格，根据每次实验所需的总量配置，多余抗体可以保存在-20°C。

Strip # 8 孔条数量	Capture Antibody (µl) 捕获抗体	Solution A (ml) 试剂 A
2	17	1.7
4	33	3.3
6	50	5.0
8	66	6.6
10	82	8.2
12	100	10.0

2. **Prepare Standard Dilutions （标准品稀释方法）**: The recommended standard range is 0.8-50 ng/ml. Dilute one vial of HMGB1 Standard with 950 µl of Sample/Standard Dilution Buffer (Solution B) - 100 ng/ml. Prepare serial dilutions of the standard by mixing 200 µl of the 100 ng/ml standard with 200 µl of Solution B - 50 ng/ml. Then repeat this procedure to make six more serial dilutions of standard - 25, 12.5, 6.25, 3.1, 1.6, and 0.8 ng/ml solutions. Partially used 100 ng/ml standard stock ***cannot*** be saved for future assays. Discard unused, diluted standard solution. Chondrex, Inc. recommends making fresh standard and serial dilutions for each assay.

   建议使用标准品浓度范围为 0.8-50 ng/ml。用 950 µl 标准品稀释液试剂 B 稀释一管 HMGB1 标准品, 充分溶解备用，浓度为 100 ng/ml。取出 7 支洁净的试剂管，分别标注 1，2，3，4，5，6，7，8，按如下图进行稀释，每管加入 200 µl 标准品稀释液试剂 B， 然后在第一管中加入 200 µl 100 ng/ml 的标准品，混匀后，再从第一管中取出 200 µl 加入到第二管中，以此类推至第七管。第八管为标准品稀释液试剂 B，作为阴性对照使用。多余的稀释过的标准品**不能**再保存使用。



3. **Prepare Sample Dilutions （样本稀释方法）**: Centrifuge samples at 10,000 rpm at 4°C for 3 minutes to remove insoluble materials and lipids and use the supernatant as samples. Dilute samples at least 1:1 with an equal volume of Solution B. For example, take 100 µl of a serum, and mix with 100 µl of Solution B.

   收集样本后，10,000 rpm 4°C 离心 3 分钟, 取上清，用试剂 B 以 1：1 的比例稀释。例如，将 100 µl 血清上清液和 100 µl 试剂 B 混匀使用。

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4. **Prepare Detection Antibody（检测抗体）**: Reconstitute one vial of Detection Antibody with 50 µl of distilled water to make a stock solution. Dilute the 50 µl of detection antibody stock solution in 5 ml of Detection Antibody Dilution Buffer (Solution C). If planning to use less, the remaining stock solution can be stored in its original vial at -20°C.

   用 50 µl 双蒸馏水溶解检测抗体备用。用检测抗体稀释液试剂 C 将 100X 检测抗体稀释成 1X 的检测抗体工作液。例如，50 µl 的 100x 检测抗体需要用 5 ml 试剂 C 进行稀释。根据以下表格配置实验所需总量的检测抗体，多余抗体可以保存在-20°C。

Strip # 8 孔条数量	Detection Antibody (µl) 检测抗体	Solution C (ml) 试剂 C
2	8	0.8
4	17	1.7
6	25	2.5
8	33	3.3
10	42	4.2
12	50	5.0

5. **Dilute Wash Buffer （稀释洗涤液）**: Dilute 50 ml of 20X wash buffer in 950 ml of distilled water (1X wash buffer). Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

   用 950 ml 的蒸馏水稀释 50 ml 20x 的浓缩洗涤液至 1X 洗涤液。用 1X 洗涤液洗板至少 3 次。最后一次洗涤后，在滤纸上将板内残留液体拍出，确保板内无残留液体。板条也不能长时间放置室温导致干透。

6. **Add Standards, Samples, and Detection Antibody （加入标准品，样本和检测抗体）**: Vortex standards, samples, and detection antibody tubes well. Add 50 µl of Solution B (blank), standards, and samples to appropriate wells. Add 50 µl of diluted detection antibody solution to all wells. Mix all wells by pipetting or use a plate shaker. Cover the plate with a plate sealer and incubate at 37°C for 1 hour, then transfer plate to 4°C overnight.

   将标准品，样本和检测抗体管振荡混匀， 在相应的孔中分别加入 50 µl 试剂 B（阴性对照），50 µl 标准品和 50 µl 样本。再在每孔中加入 50 µl 1x 检测抗体，充分混匀，盖上封板膜，37°C 孵育 1 小时， 然后将板转移到 4°C 孵育过夜。

7. **Wash （洗涤）**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

   用 1X 洗涤液洗涤板条至少 3 次，每孔 300 µl.可采用手动洗涤或者自动洗板机洗涤。最后一次洗涤后，在滤纸上将板内残留液体拍出，确保板内无残留液体。板条也不能长时间放置室温导致干透。

8. **Add Streptavidin Peroxidase（加酶结合物）**: Dilute one vial of Streptavidin Peroxidase in 10 ml of Streptavidin Peroxidase Dilution Buffer (Solution D). If planning to use less, the remaining stock solution can be stored in its original vial at -20°C. Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 30 minutes.

   将 200X 辣根过氧化物酶标记链霉亲和素（streptavidin peroxidase ）用试剂 D 稀释成 1X streptavidin peroxidase 工作液。例如，用 10 ml 的试剂 D 稀释一管 streptavidin peroxidase。根据以下表格配置实验所需总量的 streptavidin peroxidase 工作液体，多余 200X streptavidin peroxidase 可以保存在-20°C。每孔中加入 100 µl 1X streptavidin peroxidase 工作液，盖上封板膜，室温孵育 30 分钟。

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Strip # 8 孔条数量	Streptavidin Peroxidase (µl) 辣根过氧化物酶标记链霉亲和素	Solution D (ml) 试剂 D
2	9	1.8
4	17	3.4
6	25	5.0
8	33	6.6
10	41	8.2
12	50	10.0

9. **Wash （洗涤）**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

   用 1X 洗涤液洗涤板条至少 3 次，每孔 300 µl.可手动洗涤或者自动洗板机洗涤。最后一次洗涤后，在滤纸上将板内残留液体拍出，确保板内无残留液体。板条也不能长时间放置室温导致干透。

10. **Add TMB (加 TMB 显色)**: Use new tubes when preparing TMB. Dilute one vial of TMB with 10 ml of Chromogen Dilution Buffer just prior to use. Add 100 µl of TMB solution to all wells immediately after washing the plate. Incubate for 30 minutes at room temperature. If planning to use less, the remaining stock solution can be stored in its original vial at -20°C.

    使用前将 50X TMB 用稀释液 CDB 稀释成 1X TMB 工作液， 每孔加入 100 µl 1XTMB 显色液, 室温孵育 30 分钟。根据以下表格配置实验所需总量的 1X TMB 工作液体，多余 50X TMB 可以保存在-20° 。

Strip # 8 孔条数量	TMB (µl) 显色剂	Chromogen Dilution Buffer (ml) 显色剂稀释液
2	34	1.7
4	66	3.3
6	100	5.0
8	132	6.6
10	164	8.2
12	200	10.0

11. **Stop （加终止液）**: Add 50 µl of 2N sulfuric acid (Stop Solution) to each well.

    每孔加入 50 µl 终止液终止反应。

12. **Read Plate （读板）**: Read the OD values at 450 nm (a 630 nm filter can be used as a reference) immediately. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution.

    终止后请及时用酶标仪在 450 nm 波长测量各孔的光密度（OD 值）, 以 630 nm 为矫正波长。 如果样本浓度过高，其 OD 值高于标准曲线上限，应适当稀释后重测，确保稀释后 OD 值在标准曲线范围内。

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16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
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www.chondrex.com
support@chondrex.com

CALCULATING RESULTS 结果分析

1. Average the duplicate OD values for the blank, standards, and samples.

   计算空白孔（阴性对照），标准品和样本复孔的平均OD值。

2. Subtract the averaged blank (B) OD value from the averaged standard and sample OD values.

   从标准品和样本的平均OD值中减去空白孔的OD值。

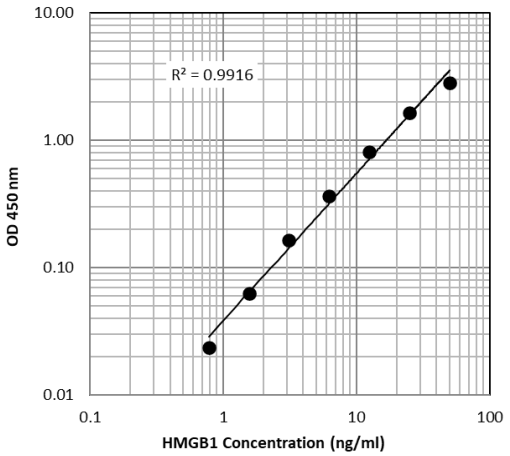
3. Plot the OD values of standards against the concentration of HMGB1 (ng/ml) using a log scale. Figure 1 shows a typical standard curve where the HMGB1 range is from 0.8-50 ng/ml.

   以标准品HMGB1的浓度的半对数为横坐标，OD值为纵坐标，绘制标准曲线图。图1显示典型的标准曲线图，其HMGB1的浓度范围为0.8-50 ng/ml。

4. The concentration of HMGB1 (ng/ml) in samples can be calculated using regression analysis. Multiply the results by the dilution factors (usually 2 without extra dilution). For additional assistance, please download a sample calculation worksheet from www.chondrex.com.

   样本中HMGB1浓度（ng/ml）可以用通过回归分析计算得出。将结果乘以稀释倍数 （如果没有额外稀释，通常稀释倍数为2）。如有疑问，请在Chondrex公司主页www.chondrex.com下载样本计算模板。

Figure 2 - A Typical Standard Curve for the HMGB1 Detection ELISA Kit



VALIDATION DATA 验证数据

Table 1 - Reproducibility Data for the HMGB1 Detection ELISA Kit 重复性

Human Serum 人血清板内和板间变异系数

Test At	2 ng/ml	7.5 ng/ml	30 ng/ml
Intra-Assay CV (%)	2.7	3.4	3.6
Inter-Assay CV (%)	1.3	5.1	3.1

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Mouse Serum 小鼠血清板内和板间变异系数

Test At	2 ng/ml	7.5 ng/ml	30 ng/ml
Intra-Assay CV (%)	1.4	3.2	8.1
Inter-Assay CV (%)	3.7	4.5	0.9

Recovery 准确度

Species	Averaged Recovery Results
Human (5 sera) 人血清	101 ± 25.1%
Mouse (5 sera) 小鼠血清	104 ± 8.3%

Specificity: Average cross reactivity with bovine HMGB2 is 12.4%.

特异性：与牛血清中的HMGB2交叉反应率为12.4%。

TROUBLESHOOTING 常见问题及解决方法

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s ELISA FAQ for more information.

关于ELISA测定中可能出现的常见问题及解决方法请参考Chondrex公司ELISA FAQ.

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