



Human CALML5 ELISA Kit

Cat. No. KIT11783

Lot No. CW19JU0701

Note: Please read this instruction manual carefully before using the product (the printed version with the product shall prevail). Different batches of reagents cannot be mixed.

Assay range: 23.44-1500 pg/mL

Sensitivity: 4.57 pg/mL

Intended Use: The kit has been verified by high purity Human CALML5 recombinant protein. The use of this kit for natural samples need to be validated by the end user due to the complexity of natural targets and unpredictable interference.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.
Tel: +86-400-890-9989 (Global), +1-215-583-7898 (USA), +49(0)6196 9678656 (Europe)
Website: <http://www.sinobiological.com>

PRINCIPLE OF THE ASSAY

The principle of this ELISA kit is based on the Sandwich Enzyme-Linked Immunosorbent Assay (ELISA). The 96-well strip plate is precoated with a monoclonal antibody specific for Human CALML5. Standards and Human CALML5 present in the sample is bound by the immobilized antibody. After washing, a horseradish peroxidase conjugated anti-Human CALML5 antibody is added, producing an antibody-antigen-antibody "sandwich complex". Following a wash to remove any unbound antibody-enzyme reagent, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Human CALML5 bound in the initial step. The color development is stopped and the intensity of the color is measured at 450 nm.

KIT CONTENTS AND STORAGE

Please use the kit before the expiration date.

Components	Amount	Preparation instructions	storage
Pre-coated microplate	1plate (96 tests)	Take the microplate strips as needed, and put the unused strips back to the vacuum bag. It is best to vacuumize them.	Vacuum storage can store at 2-8°C until expiration date and opened package store at 2-8°C for one month.
Standard	1 bottle	Add 1 mL of 1 × Dilution Buffer to the lyophilized standard bottle, briefly vortex to mix completely and prepare a standard stock solution.	The unopened lyophilized standard can be stored at 2-8°C until expiration date. Once the standard is redissolved, the liquid standard should be aliquoted and stored at -20°C to -80°C, please use it as soon as possible.
Detection Antibody	1 vial	Dilute at 1:1000 with 1×dilution buffer for 10 minutes before use. Dilute fresh as needed.	Primary liquid are stable at 2-8°C until expiration date. To be reconstituted, the working fluid is used within the working day and discard. So dilute fresh as needed.
20 × Dilution Buffer	1 bottle	If crystals have formed in the 20 × concentrated solution, bring to room temperature and mix until dissolved. Dilute the 20 × concentrated solution to 1 × working solution with deionized water. For example, make 400 ml of 1 × Wash Buffer by adding 20 mL of 20× Wash Buffer to 380 mL of deionized water. Dilute fresh as needed.	
20 × Wash Buffer	1 bottle		
Color Reagent A	1 bottle	Color Reagents A and B should be mixed together in equal volumes within 10 minutes before use. Take care not to contaminate the Color Reagent. If the mixed color reagent is blue, DO NOT USE.	
Color Reagent B	1 bottle		
Stop Solution	1 bottle	Dilute acid. Use directly according to the use volume. Pay attention to safety when using.	

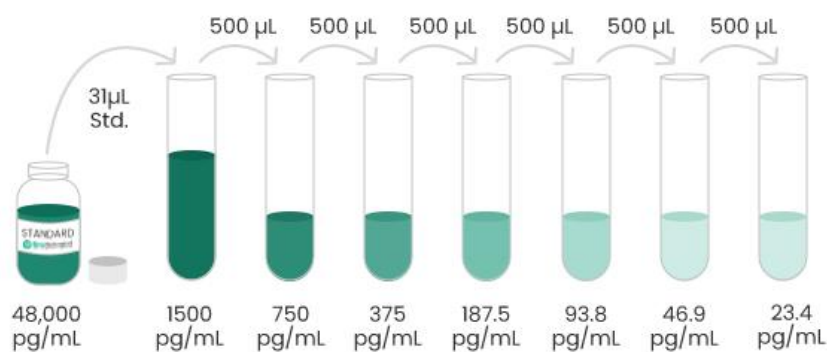
ASSAY PROCEDURE

1. Plate Set-up

- Bring all reagents to room temperature (22-28°C) equilibration (at least 30 minutes) before use. If crystals have formed in buffer solution, warm to room temperature and mix gently until the crystals have completely dissolved.
- Determine the number of wells for the assay run. Remove unused microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
- Add 300 μ L 1 $\times$ Wash Buffer to each well and let stand for about 2 minutes. Aspirate or dump the liquid and pat dry on a paper towel, wash twice in this way.

2. Incubation with standard and samples [Volume: 100 μ L Time: 2 hours]

- Make standard curve: Prepare 1000 μ L of the 1500 pg/mL top standard by adding 31 μ L of the standard stock solution in 969 μ L of 1 $\times$ Dilution Buffer. Perform six two-fold serial dilutions of the 1500 pg/mL top standard in 6 separate tubes using 500 μ L 1 $\times$ Dilution Buffer as the diluents: after mixing the 1500 pg/mL top standard, pipette 500 μ L into the next tube, and so on. 1 $\times$ Dilution Buffer serves as the zero standard (0 pg/mL). Ensures each assay has a standard curve. DO NOT USE the standard curve on other plates or other days.



- Add 100 μ L standard and your test samples per well. Cover/seal the plate and incubate for 2 hours at room temperature.
- Add 300 μ L 1 $\times$ Wash Buffer to each well and let stand for about 2 minutes. Aspirate or dump the liquid and pat dry on a paper towel, wash wells 3 times in this way. Improper washes may lead to falsely elevated signals and poor reproducibility.

3. Incubation with Secondary Antibody [Volume: 100 μ L Time: 1 hour]

- Add 100 μ L of detection antibody working solution into each well, mix gently.
- Cover/seal the plate and incubate for 1 hour at room temperature.
- Removal the liquid in the wells and repeat the aspiration/wash as in Step 2.

4. Incubation with Substrate [Volume: 100 μ L Time: about 20 minutes]

- Add 100 μ L of Substrate Solution (the mixture of Color Reagents A and B) to each well, mix gently.
- Incubate for 20 minutes at room temperature. Protect from light. (According to the color of sample and the control antibody, the chromogenic time should be shortened or prolonged.)

5. Stop reaction

- Add 100 μ L of Stop Solution to each well.
- Tap gently the plate to ensure it is well mixed.

6. Absorbance Reading

- Read absorbance of the entire plate at 450nm wavelength within 10 minutes after adding the stop solution.

ASSAY PROCEDURE SUMMARY

1. Plate set-up

Wash 2 times



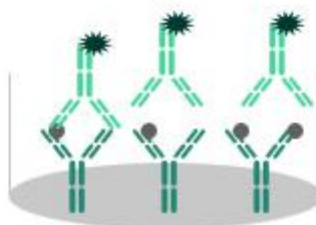
2. Incubation with standard and samples

Add 100 μ L standards or samples
Incubate 2 hours, RT



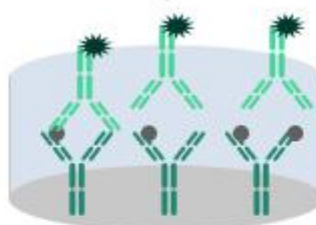
3. Incubation with Secondary Antibody

Wash 3 times
Add 100 μ L Detection Antibody solution
Incubate 1 hour, RT



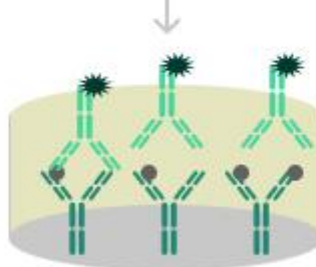
4. Incubation with Substrate

Wash 3 times
Add 100 μ L Substrate Solution
Incubate 20 min, RT, in the dark



5. Stop reaction

Add 100 μ L Stop Solution



6. Absorbance Reading

Read absorbance at 450nm
within 10 minutes

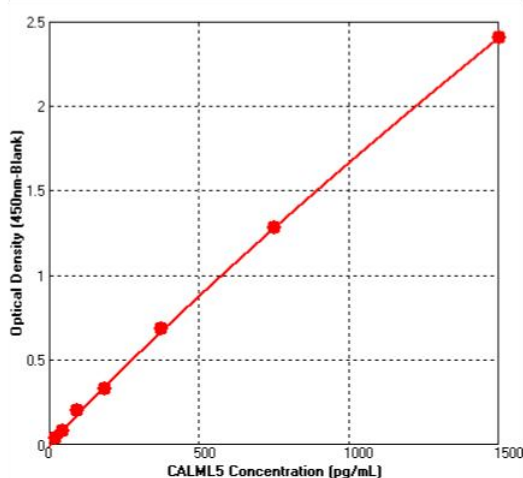
CALCULATION OF RESULTS

1. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
2. Calculate the mean absorbance for each standard and sample, subtract average zero standard optical density.
3. The data been calculated by 4-parameter logistics curve-fitting algorithm.

TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be run with each assay.

Concentration (pg/mL)	Zero standard subtracted OD-Blank
23.44	0.042
46.88	0.081
93.75	0.200
187.5	0.328
375	0.683
750	1.280
1500	2.407



PERFORMANCE CHARACTERISTICS

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 three separate assays to assess inter-assay precision.

	Intra -assay Precision			Inter -assay Precision		
Sample	1	2	3	1	2	3
N	20	20	20	3	3	3
Mean (pg/mL)	64	203	1126	68	192	1144
SD	3.451	11.740	33.274	0.43	11.39	25.66
CV (%)	5.4%	5.8%	3.0%	0.6%	5.9%	2.2%

Recovery: The recovery of Human CALML5 spiked to different levels throughout the range of the assay in related matrices was evaluated.

Sample	Average % Recovery	Range
cell culture supernates (n=4)	111	100-120%

Linearity:

		cell culture supernates
1:2	recovery of detected	113%
1:4	recovery of detected	115%
1:8	recovery of detected	90%
1:16	recovery of detected	92%

Sensitivity: 4.57 pg/mL. Which was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity: This assay recognizes recombinant human CALML5. The factors listed below were prepared at 50 ng/mL in dilution buffer and assayed for cross-reactivity. No cross-reactivity was observed.

Recombinant human			
IL1	IL2	IL4	IL6
IL8	IL10	IL33	PDGF
VEGF	TNF- α	IFN- γ	MIP-1 β
TIMP1	TIMP2	GM-CSF	SCF
CALML3	PLK1		

Interference: Preparations of PLK1 at 15 ng/mL in a mid-range recombinant human CALML5 control were assayed for interference. No significant interference was observed.

PRECAUTIONS

1. This kit is **for research use only** and is not for use in diagnostic or therapeutic procedures.
2. The kit should not be used beyond the expiration date.
3. Do not mix reagents from different lots.
4. The kit is designed and tested to detect the application which shown in the manual. The use of this kit for other purpose should be verified carefully by the end user.

SAFETY INSTRUCTIONS

1. The Stop Solution provided with this kit is an acid solution. Take care when using the reagent to avoid the risks.
2. All biological materials should be handled and discarded as potentially hazardous following local laws and regulations.
3. Personal protective equipment such as lab coats, gloves, surgical masks and goggles are necessary in experiments for safety reasons.

TECHNICAL TIPS

1. Bring all reagents and samples to room temperature before use.
2. Samples should be thawed completely and mixed well prior to analysis. Avoid repeated freeze-thaw cycles of frozen samples.
3. Use a new disposable reagent reservoir and new disposable pipette tips for each transfer to avoid cross-contamination.
4. Read the absorbance of each well within 10 minutes after adding the stop solution.



人钙调蛋白样蛋白 5 酶联免疫试剂盒

货号: KIT11783

批号: CW19JU0701

备注: 请在使用产品前仔细阅读本使用说明书（以随货打印版为准）。不同批次试剂不可混用

线性范围: 23.44-1500 pg/mL

灵敏度: 4.57 pg/mL

预期用途: 本试剂盒由高纯度人 CALML5 重组蛋白验证。由于其他样本的复杂性和不可预测的干扰，建议用户摸索试剂盒对天然样本的最佳检测条件。

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检测原理

ELISA 试剂盒的原理基于双抗体夹心酶联免疫分析技术。Human CALML5 特异性单克隆抗体预先包被在孔板条上。标准品或样品加入孔中，样品中的 Human CALML5 被固定化抗体捕获。孵育后洗涤孔板，加入辣根过氧化物酶标记的抗 Human CALML5 的抗体，产生抗体-抗原-抗体“三明治复合体”。洗涤除去未结合的抗体后，加入 TMB 底物溶液，液体颜色与 Human CALML5 的结合量成正比，然后加入终止液停止反应，可在 450nm 下测量液体颜色深浅。

试剂盒组分和保存条件

请在有效期内使用试剂盒。

组分	规格	准备说明	保存条件
酶标板	1 板 (96 孔板)	根据需要取出酶标条，并将未使用的条放回真空袋中。最好将它们抽真空。	真空储存 2-8℃保存至保质期，拆封后 2-8℃保存 1 个月。
标准品	1 瓶	加入 1 mL1×稀释缓冲液到冻干标准瓶中，短暂涡旋以完全混合，制备标准品溶液。	未开封冻干标准品可在 2-8℃保存，直至保质期。标准品一旦复溶，应分装并保存在-20℃到 -80℃，请尽快用完。
检测抗体	1 管	用 1×稀释缓冲液以 1:1000 稀释，使用前 10 分钟配置。 根据需要现用现配。	原液可在 2-8℃下保持稳定，直至保质期。 工作液请现用现稀释，并于当日用完，剩余请丢弃。
20 × 稀释缓冲液	1 瓶	如果在 20×浓缩溶液中形成晶体，则恢复至室温并混合直至溶解。用去离子水将 20×浓缩溶液稀释为 1×工作溶液。例如，将 20 mL 20×洗涤缓冲液添加到 380 mL 去离子水中，制成 400 mL 1×洗涤缓冲液。 根据需要现用现配。	
20 × 洗涤缓冲液	1 瓶		
显色液 A	1 瓶	显色液 A 和 B 应在使用前 10 分钟内等量混合在一起。注意不要污染显色液。如果显色液是蓝色的， 请勿使用。	
显色液 B	1 瓶		
终止液	1 瓶	稀酸。根据使用量直接使用。使用时注意安全。	

实验流程

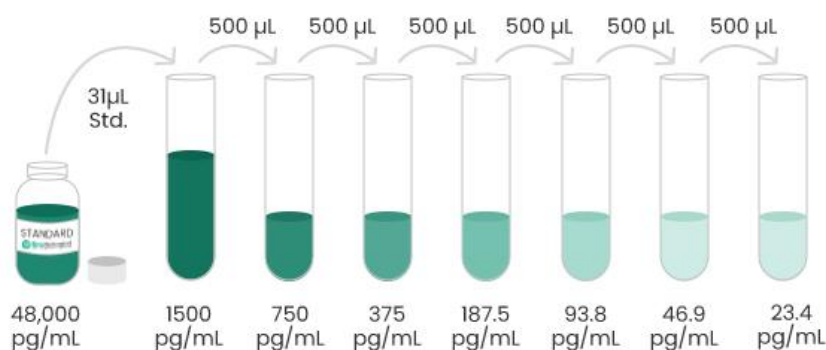
1. 准备酶标板

- 使用前将所有试剂置于室温（22-28℃）平衡状态（至少30分钟）。如果在缓冲溶液中形成晶体，温热至室温并轻轻混合直至晶体完全溶解。
- 确定测定运行的孔数。从板框上取下未使用的酶标条，将它们放回装有干燥剂包的铝箔袋中，然后重新密封。
- 每孔加入300 μL 1 $\times$ 洗涤缓冲液，静置约2分钟。吸出或倾倒液体并在纸巾上拍干，以这种方式清洗两次。

2. 标准品和样品一起孵育 [体积: 100 μL 时间: 2小时]

- 制作标准曲线：在969 μL 1 $\times$ 稀释缓冲液中加入 31 μL 标准储备溶液，制备 1000 μL 1500 pg/mL 最高浓度标准品。使用 500 μL 1 $\times$ 稀释缓冲液作为稀释剂，在6个单独的试管中对 1500 pg/mL 最高浓度标准品进行六次两倍系列稀释：在混合1500 pg/mL 最高浓度标准品后，将500 μL 移液器移入下一个试管中，依此类推上。1 $\times$ 稀释缓冲液用作零标准(0 pg/mL)。

确保每个测定都有一条标准曲线。不要在其他板上或其他日期使用标准曲线。



- 每孔加入100 μL 标准品和待测样品。盖上或密封板并在室温下孵育2小时。
- 每孔加入300 μL 1 $\times$ 洗涤缓冲液，静置约2分钟。吸出或倾倒液体并在纸巾上拍干，以此方式洗板3次。注意：洗涤不当可能会导致信号错误升高和重现性差。

3. 二抗孵育 [体积: 100 μL 时间: 1小时]

- 每孔加入100 μL 检测抗体工作液，轻轻混匀。
- 盖上或密封板并在室温下孵育1小时。
- 去除孔内液体并重复步骤2中的抽吸、洗涤。

4. 底物孵育 [体积: 100 μL 时间: 约20分钟]

- 每孔加入100 μL 底物溶液(显色液 A和B的等量混合物)，轻轻混匀。
- 室温下孵育20分钟。避光。(根据样品和对照抗体的颜色，显色时间应缩短或延长。)

5. 中止反应

- 每孔加入100 μL 终止液。
- 轻轻敲击孔板以确保其充分混合。

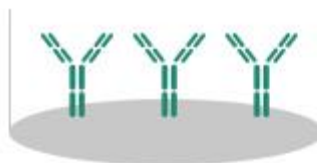
6. 吸光度读值

- 加入终止液后10分钟内，在450nm波长处读取整个板的吸光度。

实验流程汇总简图

1. 准备酶标板

洗板2次



2. 孵育标准品和样品

加入100 μL 标准品或样品
室温孵育2小时



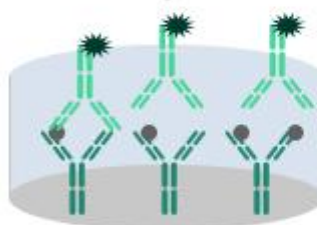
3. 孵育二抗

洗板3次
加入100 μL 检测抗体
室温孵育1小时



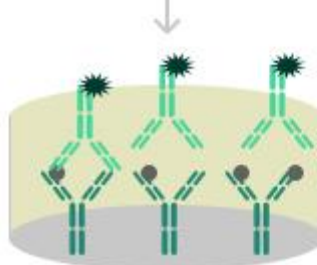
4. 孵育底物

洗板三次
加入100 μL 底物溶液
室温避光孵育20分钟



5. 终止

加入100 μL 终止液



6. 读取吸光度值

10分钟内读取450nm的光吸收值

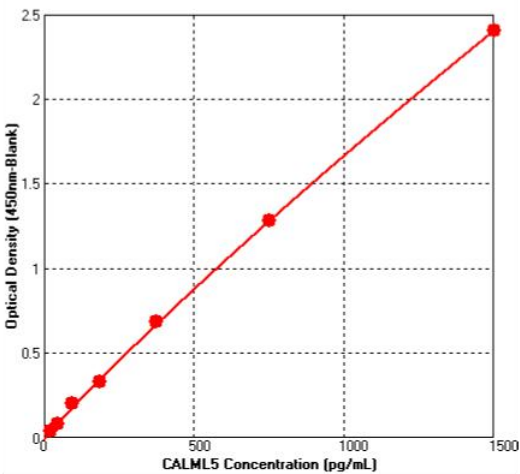
结果计算

- 1. 如果样品值高于最高标准，稀释样品并重新测定。
- 2. 计算每个标准品和样品的平均吸光度，减去空白对照平均标准光密度。
- 3. 采用四参数曲线拟合算法计算数据。

示例数据

下标准曲线图仅供参考，应以同次实验标准品所绘标准曲线计算标本含量。

Concentration (pg/mL)	Zero standard subtracted OD-Blank
23.44	0.042
46.88	0.081
93.75	0.200
187.5	0.328
375	0.683
750	1.280
1500	2.407



性能特点

精密度:

测定板内精密度：三个已知浓度的样品在一块板上测试了 20 次，以评估测定内的精确度。

测定板间精密度：在五个单独的测定中测试三个已知浓度的样品，以评估测定间的精确度。

	Intra -assay Precision			Inter -assay Precision		
Sample	1	2	3	1	2	3
N	20	20	20	3	3	3
Mean (pg/mL)	64	203	1126	68	192	1144
SD	3.451	11.740	33.274	0.43	11.39	25.66
CV (%)	5.4%	5.8%	3.0%	0.6%	5.9%	2.2%

回收率:评估了在相关基质中在整个测定范围内加标至不同水平的 Human CALML5 的回收率。

Sample	Average % Recovery	Range
cell culture supernates (n=4)	111	100-120%

线性关系:

		cell culture supernates
1:2	recovery of detected	113%
1:4	recovery of detected	115%
1:8	recovery of detected	90%
1:16	recovery of detected	92%

灵敏度: 4.57 pg/mL. 将 2 倍 SD 值加入到 20 个空白对照的平均光密度中, 计算相应浓度

特异性: This assay recognizes recombinant human CALML5. The factors listed below were prepared at 50 ng/mL in dilution buffer and assayed for cross-reactivity. No cross-reactivity was observed.

Recombinant human			
IL1	IL2	IL4	IL6
IL8	IL10	IL33	PDGF
VEGF	TNF- α	IFN- γ	MIP-1 β
TIMP1	TIMP2	GM-CSF	SCF
CALML3	PLK1		

干扰: Preparations of PLK1 at 15 ng/mL in a mid-range recombinant human CALML5 control were assayed for interference. No significant interference was observed.

注意事项

1. 本产品仅用于科研, 不能用于临床诊断或治疗。
2. 试剂盒必须在保质期内使用。
3. 不允许混用来自不同试剂盒和不同批次号的试剂。
4. 本产品仅能够应用于检测说明书中标注的靶点抗原与样本。其它应用需经使用者设计验证后, 根据结果评估使用的可靠性与准确性。

安全须知

1. 本试剂盒提供的终止液是酸性溶液。使用试剂时要小心, 避免风险。
2. 所有生物样本均具有潜在生物安全风险, 使用者应严格按照当地法律和相关规定操作处理和丢弃样本。
3. 出于安全原因, 操作者应穿戴个人防护装备, 如实验服, 手套, 口罩和护目镜。

使用提示

1. 使用前应将试剂盒的所有组分和待检样本温度恢复到室温。
2. 冻存样本检测前应彻底化冻并混匀, 并注意避免反复冻融。
3. 注意在不同样本和步骤间及时更换加样槽和枪头, 避免交叉污染。
4. 读取光吸收值应在加入终止液后10分钟内完成。