Human Fas ELISA Kit

Catalog Number: KIT10217

Please read this instruction manual carefully before using the product.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.
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BACKGROUND

CD95 (APO-1/Fas) is an important inducer of the extrinsic apoptosis signaling pathway and therapy induced apoptosis of many tumor cells has been linked to the activity of CD95. is a prototype death receptor characterized by the presence of an 80 amino acid death domain in its cytoplasmic tail. This domain is essential for the recruitment of a number of signaling components upon activation by either agonistic anti-CD95 antibodies or cognate CD95 ligand that initiate apoptosis. The complex of proteins that forms upon triggering of CD95 is called the death-inducting signaling complex (DISC). The DISC consists of an adaptor protein and initiator caspases and is essential for induction of apoptosis. CD95 is also crucial for the negative selection of B cells within the germinal center (GC). Impairment of CD95-mediated apoptosis results in defective affinity maturation and the persistence of autoreactive B-cell clones. Changes in the expression of CD95 and/or its ligand CD95L are frequently found in human cancer. The downregulation or mutation of CD95 has been proposed as a mechanism by which cancer cells avoid destruction by the immune system through reduced apoptosis sensitivity. Thus, CD95 has therefore been viewed as a tumor suppressor. CD95 has been reported to be involved in the activation of NF-kappaB, MAPK3/ERK1, MAPK8/JNK, and the alternate pathways for CTL-mediated cytotoxicity. Accordingly, this protein is implicated in the pathogenesis of various malignancies and diseases of the immune system. The CD95/CD95L system was implicated in the etiology of inflammatory bowel disease (IBD) based, primarily, on the finding that CD95 is highly expressed in the intestinal epithelial cells and that epithelial apoptosis is increased in IBD.

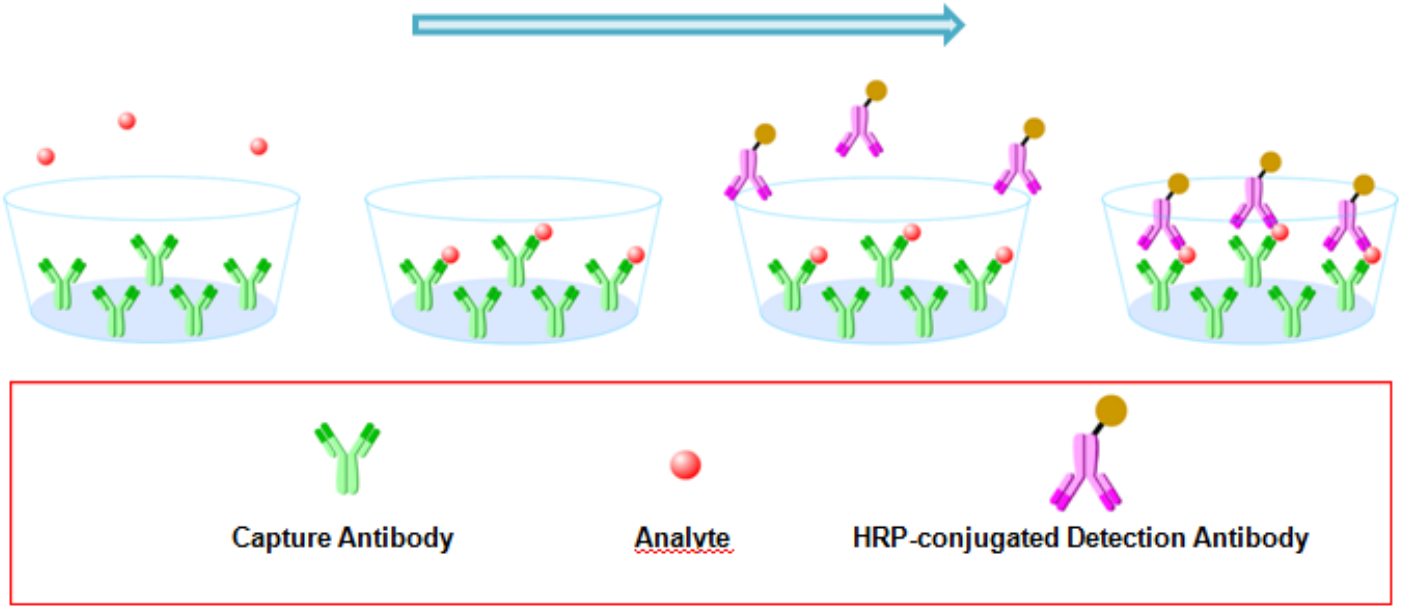
INTENDED USE

For the quantitative determination of Human Fas concentration in serum, cell culture supernates and plasma.

The use of this kit for other sample types need be validated by the end user due to the complexity of natural targets and unpredictable interference.

PRINCIPLE OF THE ASSAY

The principle of this ELISA kit is based on the solid phase sandwich enzyme immunoassay technique. A monoclonal antibody specific for Human Fas has been pre-coated onto well plate strips. Standards and samples are added to the wells and Human Fas present in the sample is bound by the immobilized antibody. After incubation the wells are washed and a horseradish peroxidase conjugated anti-Human Fas antibody is added, producing an antibody-antigen-antibody "sandwich complex". Following a wash to remove any unbound antibody a TMB substrate solution is loaded and color develops in proportion to the amount of Human Fas bound. The reaction is stopped by the addition of a stop solution and the intensity of the color can be measured at 450 nm (See schematics below).



MATERIALS PROVIDED

Human Fas Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with mouse mAb antibody against Human Fas.

Human Fas Detecion Antibody - 0.2 mg/mL of mouse mAb antibody against Human Fas conjugated to horseradish peroxidase (HRP) with preservatives.

Human Fas Standard - Recombinant Human Fas in a buffer with preservatives, lyophilized. The amount of standard is lot specific and indicated on the label of standard vial.

Wash Buffer Concentrate - 25 mL of a 20-fold concentrated solution of buffered surfactant with preservatives.

Dilution Buffer Concentrate - 8 mL of a 20-fold concentrated dilution buffer with preservatives.

Color Reagent A - 13 mL of stabilized hydrogen peroxide.

Color Reagent B - 13 mL of stabilized chromogen (tetramethylbenzidine).

Stop Solution - 8 mL of 2 N sulfuric acid.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8℃ and the kit is stable for 6 months upon receipt.</td></tr>
<tr><td rowspan="7">Opened/ Reconstituted Reagents</td><td>Diluted Wash Buffer</td><td rowspan="2">Stored for up to 1 week at 2 - 8℃</td></tr>
<tr><td>Diluted Dilution Buffer</td></tr>
<tr><td>Conjugate</td><td rowspan="3">Stored for up to 1 month at 2 - 8℃</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td><td rowspan="2">After reconstitution, store for up to 1 month at -80℃.
The reconstituted standards should be aliquoted and
avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Standard</td></tr>
<tr><td>Microplate Wells</td><td>Return unused strips to the foil pouch containing the desiccant pack and reseal along entire edge of zip-seal.
Stored for up to 1 month at 2 - 8℃</td></tr>
</table>

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Multi -channel pipette, squirt bottle, manifold dispenser, or automated microplate washer
- 500 mL graduated cylinder
- Tubes for standard dilution
- Well plate cover or seals

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 specific targets and samples shown in the manual. The use of this kit for other purpose should be verified carefully by the end user.

SAFETY INSTRUCTIONS

5. The Stop Solution provided with this kit is an acid solution. Take care when using the reagent to avoid the risk.

6. All biological materials should be handled and discarded as potentially hazardous following local laws and regulations.

7. Personal protective equipments such as lab coats, gloves, surgical masks and goggles are necessary in experiments for safety reasons.

TECHINICAL TIPS

8. Bring all reagents and samples to room temperature before use.

9. Samples should be thawed completely and mixed well prior to analysis. Avoid repeated freeze-thaw cycles of frozen samples.

10. A standard curve should be generated for each set of sample assayed. DO NOT USE the standard curves from other plates or other days.

11. Use a new disposable reagent reservoir and new disposable pipette tips for each transfer to avoid cross-contamination.

12. Read the absorbance of each well within 20 minutes after adding the stop solution.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20℃ or lower temperature. **Avoid repeated freeze -thaw cycles.**

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20℃ or lower temperature. **Avoid repeated freeze-thaw cycles. If the use of original supernate sample or low dilution (<5 fold) are necessary due to the expected low concentration of antigen supernates need be adjust to neutral pH condition before assay.**

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and storesamples at -20 °C. **Avoid repeated freeze-thaw cycles.**

Note:

The sample should be diluted to within the working range of the assay in 1× dilution buffer. The exact dilution must be determined based on the concentration of specific target in individual samples.

REAGENT PREPARATION

Bring all reagents to room temperature before use. If crystals have formed in buffer solution, warm to room temperature and mix gently until the crystals have completely dissolved.

Wash Buffer - Prepare 1× wash buffer by adding 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 400 mL of Wash Buffer.

Dilution Buffer - Prepare 1× dilution buffer by adding 5 mL of Dilution Buffer Concentrate to deionized or distilled water to prepare 100 mL of Dilution Buffer.

Detection Antibody - Centrifuge at 10,000 x g for 20 seconds. Dilute to **work concentration** of 0.5 μg/mL in Dilution Buffer before use.

Substrate Solution - Color Reagents A and B should be mixed together in equal

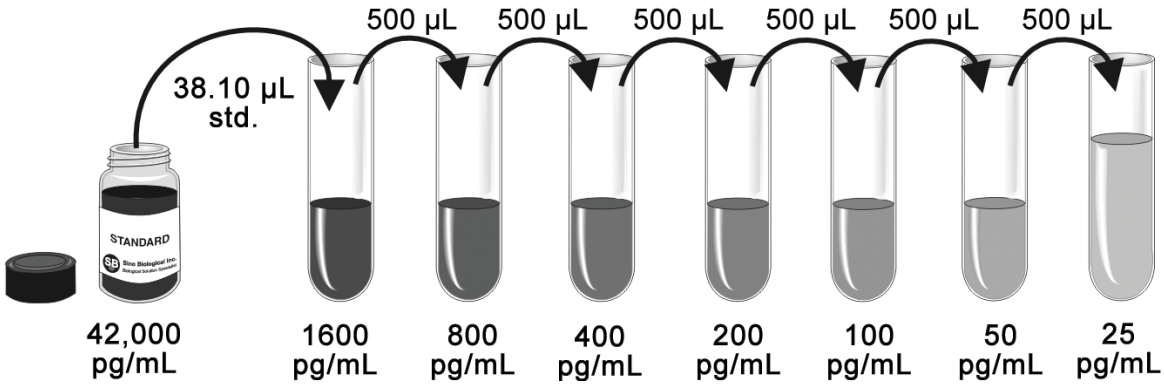
volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well. **Take care not to contaminate the Color Reagent. If the mixed color reagent is blue. DO NOT USE.**

Human Fas Standard - Reconstitute the Human Fas Standard with 1 mL of Dilution Buffer to make stock solution. Shake the vial gently until the lyophilized powder totally dissolved (**Do not turn the vial upside down**). Mix the standard to ensure complete reconstitution prior to making dilutions.

Prepare serially diluted standards as described in the following step:

Pipette 1000 µL of Dilution Buffer into the 1600 pg/mL tube. Pipette 500 µL of Dilution Buffer into the remaining tubes. Use the stock solution to produce a dilution series as the following figure. Mix each tube thoroughly before the next transfer. The 1600 pg/mL standard serves as the high standard. The 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.**

The following graph is only for demonstration purposes. The concentration of stock solution is lot specific and need be calculated with the actual amount of standard labeled on the standard vial.



ASSAY PROCEDURE

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

1. Prepare all reagents, working standards, and samples as directed in the previous

sections.

2. Remove unused microplate strips from the plate frame, return them to the foil pouchcontaining the desiccant pack, and reseal.

3. Wash each well three times with Wash Buffer (300 µL/well) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. **Complete removal of liquid at each step is essential to good performance. Remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.**

4. Add 100 µL of each serially diluted protein standard or test sample per well including a zero standard. **Ensure reagent addition is uninterrupted and completed within 15 minutes.** Cover/seal the plate and incubate for 2 hours at room temperature.

5. Repeat the aspiration/wash as in Step 3.

6. Add 100 µL of Detection Antibody in working concentration to each well. Cover/seal the plate and incubate for 1 hour at room temperature.

7. Repeat the aspiration/wash as in Step 3.

8. Add 200 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature**. Protect from light.**

9. Add 50 µL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

10. **Determine the optical density of each well within 20 minutes**, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

If samples generate values higher than the highest standard, dilute the samples and repeat the assay.

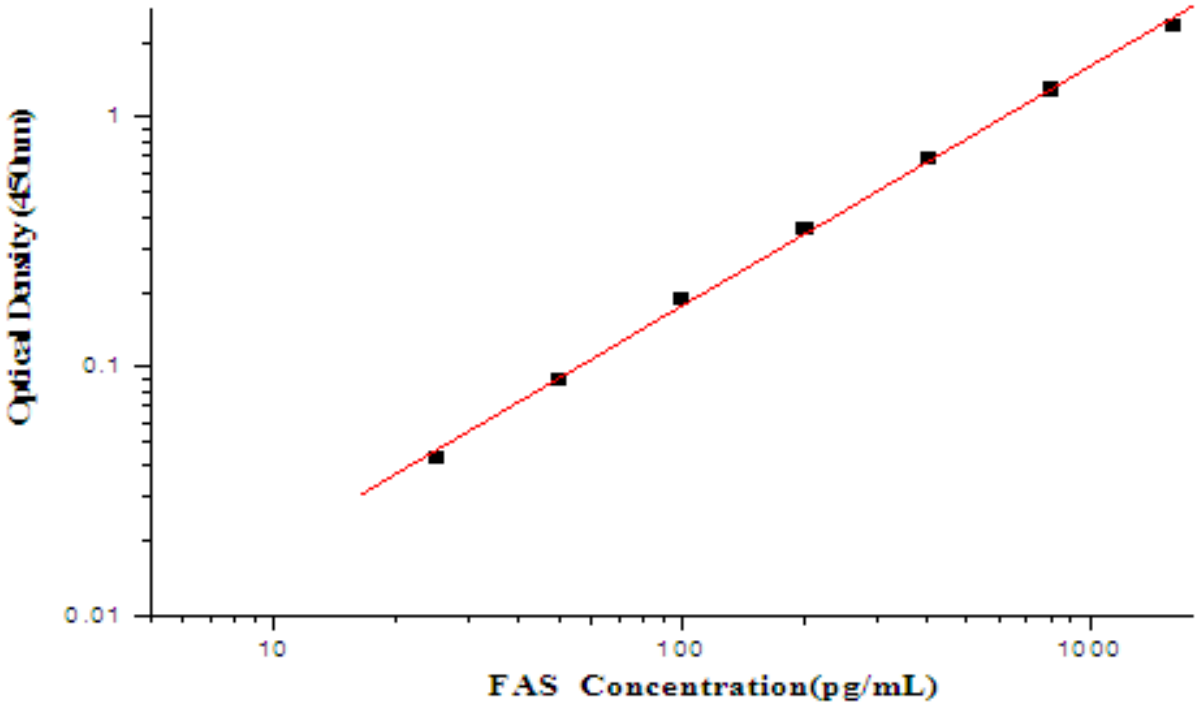
Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (O.D.) .

Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. Most graphing software can help make the curve and a four parameter logistic (4-PL) usually provide the best fit, though other equations (e.g. linear, log/log) can also be tried to see which provides the most accurate. Extrapolate the target protein concentrations for unknown samples from the standard curve plotted.

TYPICAL DATA

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

Concentration (pg/mL)	Zero standard subtracted OD
0	0
25	0.043
50	0.089
100	0.188
200	0.361
400	0.682
800	1.295
1600	2.345



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 five 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)	339	644	1169	346	658	1191
SD	9.43	16.72	42.46	4.28	18.45	39.70
CV (%)	2.8%	2.6%	3.6%	1.2%	2.8%	3.3%

RECOVERY

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

Sample	**Average % Recovery**	**Range**
Cell culture supernates (n=3)	80	78 -84%
Serum (n=3)	96	91 -102%
Plasma (n=3)	94	85 -100%

LINEARITY

		Cell culture supernates	**Serum**	**Plasma**
1:2	recovery of detected	101%	84%	82%
1:4	recovery of detected	103%	116%	118%
1:8	recovery of detected	117%	114%	118%
1:16	recovery of detected	97%	95%	106%

SENSITIVITY

The minimum detectable dose (MDD) of Human Fas is typically less than 14.6 pg/mL. The MDD was determined by adding three 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 HEK 293-expressed recombinant Human Fas produced at Sino Biologcial Inc., (Cat# 10217-H08H).

SAMPLE VALUES

The average concentration of Human FAS in 10 normal human serum is 1.78 +/- 0.71 ng/mL ranging from 0.92 to 3.11 ng/mL. The average concentration of Human FAS in 10 normal human plasma is 1.38 +/- 0.22 ng/mL ranging from 1.12 to 1.82 ng/mL. Human peripheral blood mononuclear cells (1 x 10E6 cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum, 50 μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate, stimulated with 10 μg/mL PHA for 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of natural FAS, and measured 88.71 pg/mL.

SPECIFICITY

This assay recognizes both recombinant and natural Human FAS. 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			
CNTF	EGF	FGF basic	GM-CSF
HGF	IL-1α	IL1b	IL1RA
IL1R1	IL1R2	IL2Ra	IL3RA
IL5Ra	IL2	IL3	IL-4
IL5	IL6	IL6R	IL7
IL-8	IL9	IL10	IL11
IL12	IL13	LIF	β-NGF
PTN	RANTES	SCF	SLPI
TGFB2	TNFA	TNFβ	TNFRI
TNFRII	Epo	Fas Ligand	TGF-α
FGF-4	FGF-6	CSF	IL6ST
IGF-I	IGF-II	TGF-b1	CSF2RB
MCP-1	CCL3	CCL4	OSM
TGFB2	FGF acidic	HB-EGF	γ-IFN
IL-1	IL-33	PDGF	VEGF
TIMP-1	TIMP-2	MIP-1β	
Recombinant mouse			
IL1A	IL1B	IL4	IL5
IL6	IL13	LIF	TNFa
FAS	CSF2	IL7	KITL
CCL3			
Recombinant rat			
FAS			

Preparations of the factors listed below at 50 ng/mL in a mid-range recombinant human FAS control were assayed for interference. No significant interference was observed.

Recombinant human			
CNTF	EGF	FGF basic	GM-CSF
HGF	IL-1α	IL1b	IL1RA
IL1R1	IL1R2	IL2Ra	IL3RA
IL5Ra	IL2	IL3	IL-4
IL5	IL6	IL6R	IL7
IL-8	IL9	IL10	IL11
IL12	IL13	LIF	β-NGF
PTN	RANTES	SCF	SLPI
TGFB2	TNFA	TNFβ	TNFRI
TNFRII	Epo	Fas Ligand	TGF-α
FGF-4	FGF-6	CSF	IL6ST
IGF-I	IGF-II	TGF-b1	CSF2RB
MCP-1	CCL3	CCL4	OSM
TGFB2	FGF acidic	HB-EGF	γ-IFN
IL-1	IL-33	PDGF	VEGF
TIMP-1	TIMP-2	MIP-1β	
Recombinant mouse			
IL1A	IL1B	IL4	IL5
IL6	IL13	LIF	TNFa
FAS	CSF2	IL7	KITL
CCL3			
Recombinant rat			
FAS			

TROUBLE SHOOTING

Problems	Possible Sources	Solutions
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Standard was incompletely reconstituted or was inappropriately stored	Aliquot reconstituted standard and store at -80 ℃. The reconstituted standards should be aliquoted and avoid repeated freeze-thaw cycles.
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature, timing or agitation	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	Color Reagent should be clear and colorless prior to addition to wells	Color Reagent should be clear and colorless prior to addition to wells
	Use clean tubes and pipettes tips	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples

PRECAUTIONS_中文版

注意事项：

1. 本产品仅用于研究，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CW12JL0204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。

SAFETY INSTRUCTIONS_中文版

安全提示：

1. 本试剂盒中的终止液为酸溶液,应注意小心操作。
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。

TECHINICAL TIPS_中文版

应用技巧：

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. 每次试验均需制备相应的标准曲线，不同试剂盒以及不同天的标准曲线不能混用。
4. 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。
5. 读取光吸收值应在加入终止液后二十分钟内完成。

REAGENT PREPARATION_中文版

试剂准备:

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。

1×洗涤缓冲液配制 – 如浓缩洗涤缓冲液中已形成结晶，请平衡到室温至结晶完全溶解，混匀后取 20 mL 20×浓缩洗涤缓冲液至去离子水或超纯水中，定容至 400 mL。

1×稀释缓冲液的配制 – 如果浓缩稀释缓冲液中已形成结晶，请平衡到室温至结晶完全溶解，混匀后取 5 mL 20×浓缩稀释缓冲液至去离子水或超纯水中，定容至 100 mL。

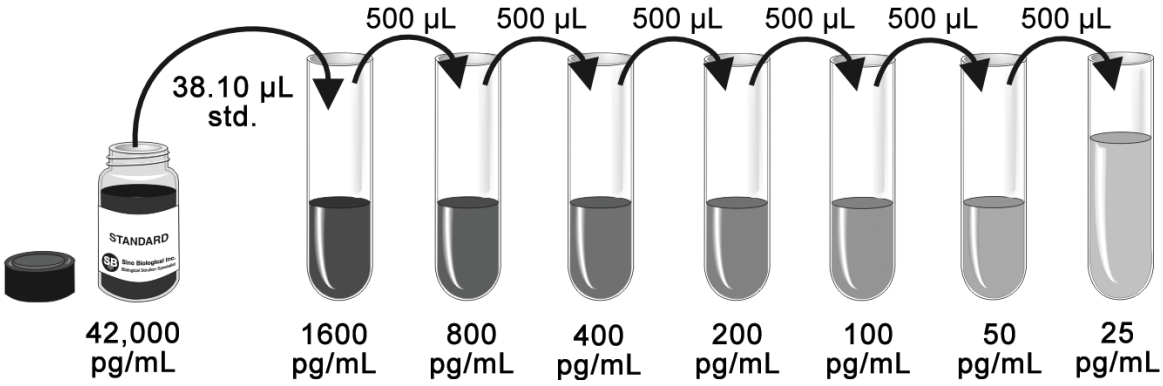
检测抗体的配制 – 使用前 10,000g 离心 20 秒，然后用 1×稀释缓冲液将酶标检测抗体稀释至工作浓度，0.5 μg/mL。

底物液的配制 – 使用前 15 分钟将显色 A 液、显色 B 液等体积混合，避光。确保底物液不被污染，如混合后的底物液已经变蓝，请勿使用。

标准品复溶 – 将 1mL 1×稀释液加入到冻干标准品的安瓶中制备标准品储液,充分溶解，混匀后（勿翻转管子）等体积分装，-80 度保存，复溶后的储液浓度应根据冻干标准品标签上的标注蛋白量进行计算。

标准曲线的制备 -取 8 个管，按照标准品浓度依次进行标记，移取 1000 μL 1×稀释缓冲液至标记为 1600 pg/mL 的离心管中，其余各管移取 500 μL，根据标准品储液浓度计算 1600 pg/mL 标准品应移取的储液体积，加至离心管中，混匀，取 500 μL 至下一标记浓度的离心管中，混匀…进行一系列倍比稀释；1600 pg/mL 为标准曲线最高点，1×稀释缓冲液为空白 (0 pg/mL)。每次试验均需制备相应的标准曲线，不同试剂盒以及不同天的标准曲线不能混用。

下图仅用于标准曲线制备范例展示，由于冻干标准品的批次差异，复溶后标准品储液的蛋白浓度不同，应根据实际浓度计算配制标准曲线所需的储液体积。



ASSAY PROCEDURE_中文版

试验流程:

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。强烈建议所有的标准品和待检样本进行双复孔测定。

1. 按前述试剂准备项准备好各种试剂、标准品和待测样本。

2. 计算检测样本所需酶标条，将酶标条从铝箔袋取出，剩余的酶标条放回铝箔袋中并封好袋口，低温保存。

3. 洗板：用 1×洗涤缓冲液(300 μL/孔)洗板三次，拍干酶标板。洗板对试验结果有重要影响，确保最后一次拍板没有洗液残留。

4. 样本孵育：加入标准品和待测样本，100 μL/孔,确保 15 分钟内完成点样，室温孵育 2 小时。

5. 洗板：弃去孔中液体，加入 1×洗涤缓冲液(300 μL/孔)洗板三次，拍干酶标板。

6. 酶标检测抗体孵育：将预先配制至工作浓度的检测抗体加入酶标板中，100μL/孔，混匀，室温孵育 1 小时。

7. 洗板：弃去孔中液体，加入 1×洗涤缓冲液(300 μL/孔)洗板三次，拍干酶标板。

8. 显色：将预先配制的底物液加入酶标板中，200 μL/孔，混匀，室温避光孵育 20 分钟。

9. 终止：加入 50 μL/孔终止液至酶标板中，轻轻震动酶标板至显色均匀。

10. 读值：20 分钟内读取 450nm 的光吸收值。

CALCULATION OF RESULTS_中文版

结果处理:

如果待测样本 OD 值超出标准曲线最高点 OD 值，需将样本进行稀释后重新测定。

取标准品、空白对照、样本的平均光吸收值，减去空白对照的平均光吸收值，得到标准品、样品的光吸收校准值。以标准品浓度为横坐标，校准后的标准品光吸收值为纵坐标绘制标准曲线。

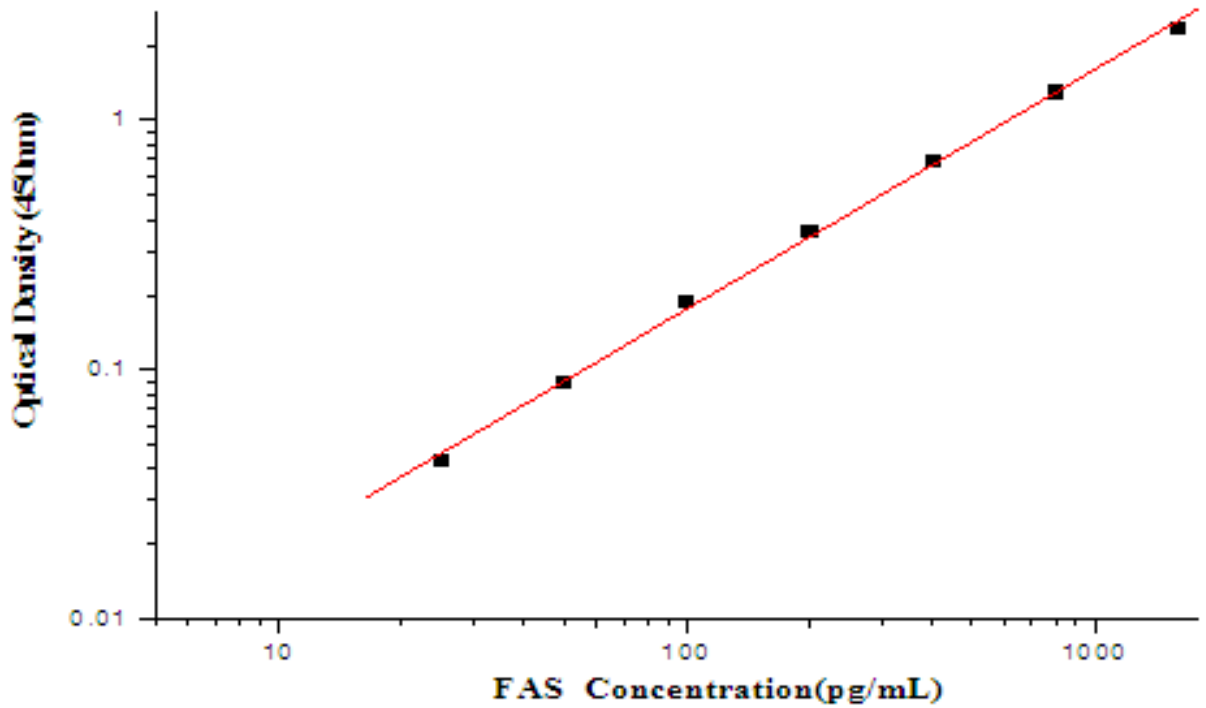
多种绘图和统计学软件可以用于辅助绘制标准曲线并进行未知样本浓度的计算。四参数拟合法往往曲线拟合效果较好，但其它方法如线性，双对数法也可能获得较好拟合结果，需要根据具体实验数据进行分析。

TYPICAL DATA_中文版

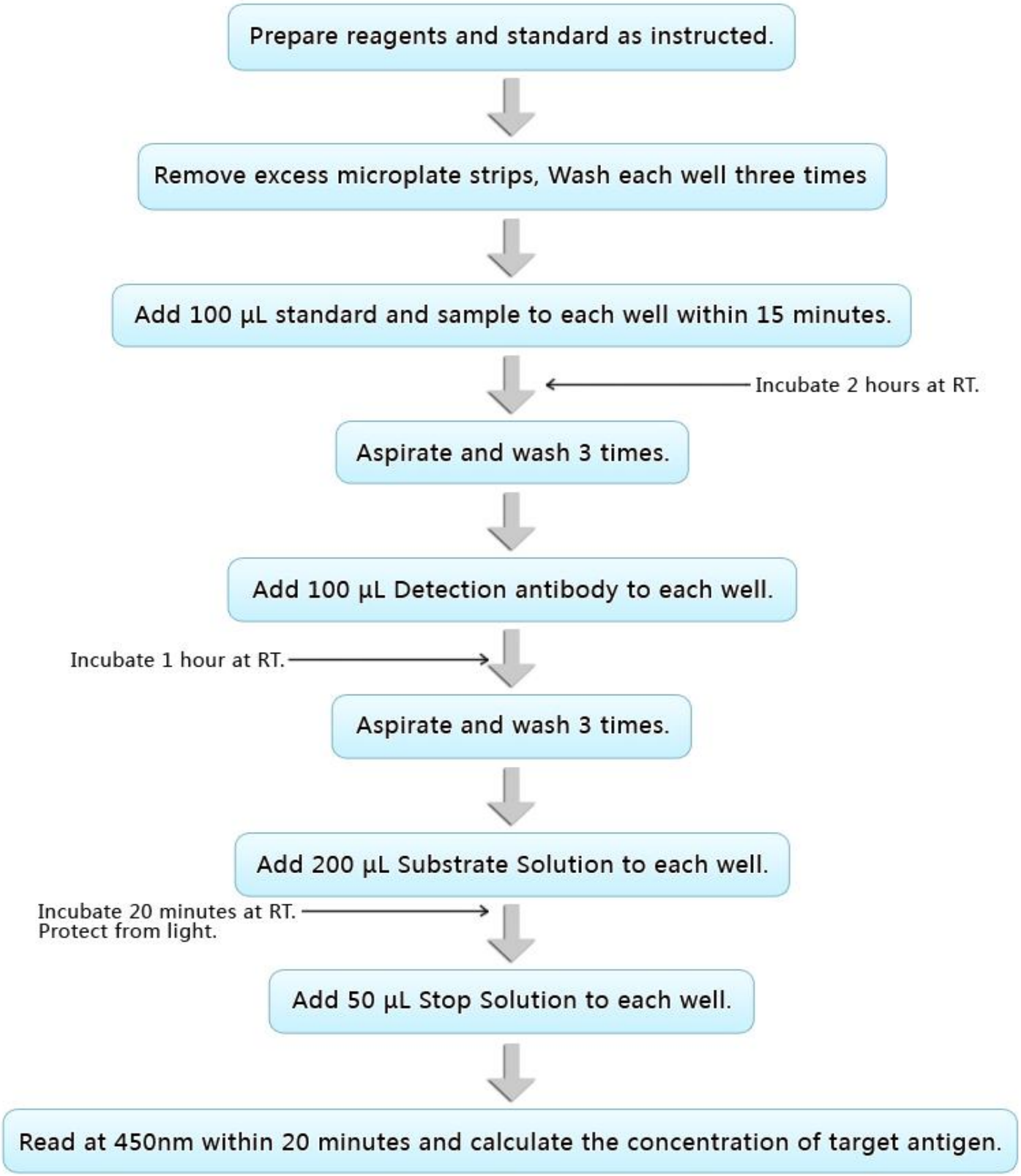
示例数据

以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
0	0
25	0.043
50	0.089
100	0.188
200	0.361
400	0.682
800	1.295
1600	2.345



ASSAY SUMMARY



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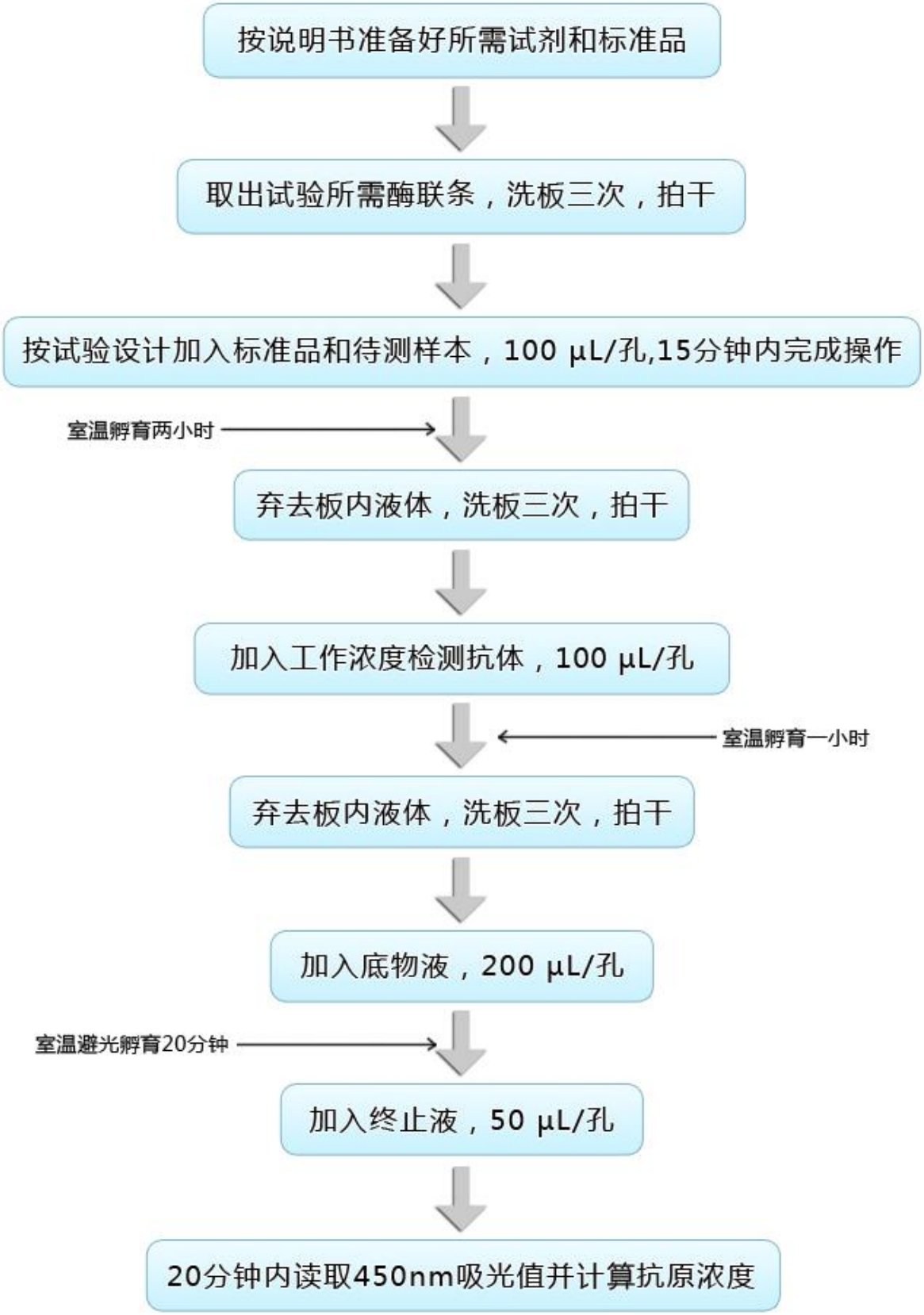
ASSAY SUMMARY_中文版

实验流程汇总简图



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