RES98-EN.02

resDetect™ BSA ELISA Kit (USP Calibrated)

Catalog Number：RES-A098

Pack Size: 96 tests

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedure

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INTENDED USE

The kit is developed for the detection and quantitative determination of BSA. The kit is calibrated using USP standard (cat # 1076192). It is used as a tool to aid in optimal purification process development and in routine quality control of in-process streams as well as final product by various biotechnological processes such as cell or tissue culture.

BACKGROUND

Most commercial formulations of serum free media contain significant amounts of albumin and transferrin either of bovine or human origin, and insulin from various species. When the intended product may be used as a therapeutic agent in humans or animals the product should be highly purified. Efforts to reduce trace media impurities to the lowest levels practical through optimal process design, qualification, and final product testing require a highly sensitive and reliable analytical method.

PRINCIPLE OF THE ASSAY

The resDetect™ BSA ELISA Kit (USP Calibrated) is used to measure the levels of BSA (Recombinant Bovine Serum Albumin) by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with anti-BSA antibody. Firstly adding standards and samples, next add the HRP-Anti-BSA Antibody to the plate. At last, load the tetramethylbenzidine (TMB) substrate into the wells and monitor a blue color. The reaction is stopped by the addition of a stop solution and the color turns yellow. The intensity of the absorbance can be measured at 450 nm and 630 nm on a microtiter plate reader. The OD Value reflects the amount of BSA (Recombinant Bovine Serum Albumin).

PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.

2. The kit is suitable for cell supernatant and serum samples.

3. Do not use reagents past their expiration date.

4. Do not mix or substitute reagents with those from other kits or other lot number kits.

5. If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay. If cell supernatant samples need step dilution, except for the final dilution with diluent, other

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intermediate dilutions can be in cell culture medium.

6. Differences in test results can be caused by a variety of factors, including laboratory operator, pipette usage, plate washing technique, reaction time or temperature, and kit storage.

7. This kit is designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed.

MATERIALS PROVIDED

Table1. Materials provided

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RES98-C01	Pre-Coated Anti-BSA Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
RES98-C02A	BSA Standard (200ng/mL)	500 µL	Liquid	2-8°C	2-8°C
RES98-C02B	BSA Standard 5 (40.5ng/mL)	500 µL	Liquid	2-8°C	2-8°C
RES98-C02C	BSA Standard 4 (13.5ng/mL)	500 µL	Liquid	2-8°C	2-8°C
RES98-C02D	BSA Standard 3 (4.5ng/mL)	500 µL	Liquid	2-8°C	2-8°C
RES98-C02E	BSA Standard 2 (1.5ng/mL)	500 µL	Liquid	2-8°C	2-8°C
RES98-C02F	BSA Standard 1 (0.5ng/mL)	500 µL	Liquid	2-8°C	2-8°C
RES98-C02G	BSA Standard 0 (0ng/mL)	500 µL	Liquid	2-8°C	2-8°C
RES98-C03	HRP-Anti-BSA Antibody	100 µL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RES98-C04	1×Sample Dilution Buffer	50 mL×2	Liquid	2-8°C	2-8°C
RES98-C05	20×Washing Buffer	25 mL	Liquid	2-8°C	2-8°C
RES98-C06	Substrate Solution	12 mL	Liquid	2-8°C	2-8°C

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RES98-C07	Stop Solution	8 mL	Liquid	2-8℃	2-8℃

Note: *It is recommended that HRP-Anti-BSA Antibody be centrifuged briefly before use to deposit liquid from the tube wall or cap to the bottom of the tube.*

SRORAGE

1. Unopened kit should be stored at 2℃-8℃ upon receiving.

2. Find the expiration date on the outside packaging and do not use reagents past their expiration date.

3. The opened kit should be stored per components table. The shelf life is 30 days from the date of opening.

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

1. Single or multi-channel micropipettes and pipette tips: need to meet 10 μL, 300 μL, 1000 μL injection requirements;

2. Orbital microtiter plate shaker：For shaking the plate in immunological steps；If there is no Orbital microtiter plate shaker, it can also be incubated in a 37℃ incubator；

3. Single or dual wavelength microplate reader with 450 nm and 630 nm filter;

4. Tubes: 1.5 mL,10 mL;

5. Timer;

6. Reagent bottle;

7. Deionized or distilled water.

PREPARATION BEFORE EXPERIMENT

1. Preparation of experimental environment

Environment In order to ensure the accuracy of the experiment, the experimental environment requires that no additional antibodies or BSA be introduced during the operation.

Please prepare a clean bench and necessary tools: Clean the operating table with 75% ethanol before the experiment, and wipe the operator's hands and arms with 75% ethanol during the experiment to avoid the introduction of additional BSA during the experiment.

2. Prepare equipment and tools：

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1) Refer to **" REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED"** to prepare the equipment, tools, reagent bottles and other utensils required for the experiment.

2) Thorough cleaning of the antibody coated plate to remove excess unreacted reagents is essential for good detection reproducibility and sensitivity. If an automatic plate washer is used to clean the labeled plate, it is recommended to use it exclusively for this experiment and to distinguish it from the buffer liquid system containing BSA to avoid the introduction of additional BSA. If you do not have an automatic board washer, you can manually clean the board with a multi-channel pipette. Thorough washing procedures typically provide a lower background, higher specific binding signals, and better precision.

REAGENT PREPARATION

Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, bring the solution back to room temperature before use.

RECOMMENDED SAMPLE PREPARATION

1. Working Solution Preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 25 mL 20×Washing Buffer with ultrapure water/deionized water to 500 mL, gently mix the required 1×WashingBuffer according to the experimental dosage.

1.2 Preparation of HRP-Anti-BSA Antibody Working Buffer:

According to experimental dosage (50 µL/well), Dilute HRP Anti-BSA Antibody to 100-fold with 1×Sample Dilution Buffer (RES98-C04). Please prepare it for one-time use only.

Please refer to the following methods to prepare the HRP-Anti-BSA Antibody Working Buffer:

Table 2. Preparation method

Tests	HRP-Anti-BSA Antibody Working Buffer	HRP-Anti-BSA Antibody	1×Sample Dilution Buffer
96 Tests	6000 µL	60 µL	5940 µL

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1.3 Prepare the Samples:

Bring the sample to room temperature and mix the sample well before adding the sample. If the sample has precipitation, it is recommended to centrifuge the sample at 1500 rpm/min for 5min and take the supernatant for detection.

If the concentration of the sample to be tested is higher than the upper limit measured by the kit, it should be diluted with 1×Sample Dilution Buffer to a linear range for testing.

Note:

a. It is recommended to do at least 2 dilutions for the sample to be tested.

b. The samples to be tested need to be spiked recovery.

c. For samples tested with this kit for the first time, interference tests are recommended.

d. RES98-C02A can be used for spiked recovery test and interference tests.

2. Add Samples

The standard (0~40.5 ng/mL) and the sample to be tested were added to the enzyme label plate in sequence, 50 µL/ well; Antibody is then added to HRP Anti-BSA Antibody Working Buffer (50 µL/ well).

Note: *All standards and samples to be tested should be on the same board and treated in the same way；*

It is recommended to set double holes for samples and standard curves to be tested.

3. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for (20℃-25℃) 1 hour at 400 rpm.

4. Washing

Thorough washing is essential to proper performance of this assay. Automated plate washing systems or manual wash procedure be selected according to your own experimental conditions.

Remove the sealing film carefully, discard the liquid in the holes, add 300 µL1×Washing Buffer to each hole, discard 1×Washing Buffer in the holes, pat the board dry on a non-fluff absorbent paper, please note that the washing buffer must be completely removed. Repeat the above cleaning steps four times.

Note: *When using the automatic washing machine, it is recommended to wash the board manually for the first time, and*

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then use the automatic washing machine for the last three times. Contaminated board washing machine needles.

5. Substrate Reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature for 20 min, avoid light.

6. Termination

Add 50 µL Stop Solution to each well and tap the plate gently to allow thorough mixing.

Note: *The color in the wells should change from blue to yellow.*

7. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 10 minutes.

Note: *To reduce the background noise, subtract the value read at* OD_{450nm} *with the value read at* $OD_{630\ nm}$.

CALCULATION OF RESULTS

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

2. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.

3. Normal range of Standard curve: $R^2 \geq 0.9900$.

4. Detection range: 0.5 ng/mL-40.5 ng/mL. If the OD value of the sample to be tested is higher than 40.5 ng/mL, the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 0.5 ng/mL, the sample should be reported.

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QUICK GUILD

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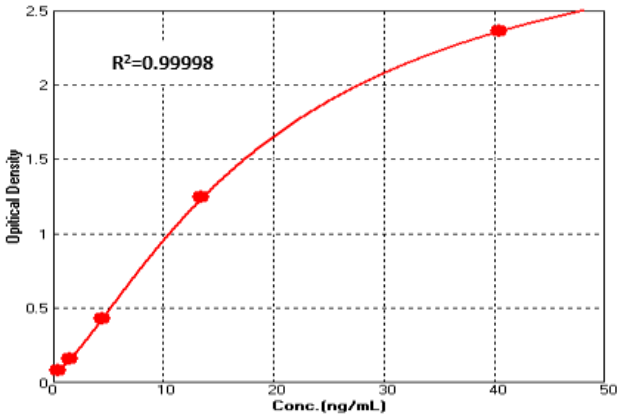
TYPICAL DATA

For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.

Standard (ng/mL)	O.D.-1	O.D.-2	O.D.-3	Average	Corrected
40.5	2.755	2.501	2.351	2.536	2.469
13.5	1.337	1.308	1.308	1.318	1.251
4.5	0.561	0.516	0.479	0.519	0.452
1.5	0.262	0.228	0.226	0.239	0.172
0.5	0.133	0.146	0.148	0.142	0.075
0	0.072	0.066	0.063	0.067	/



SENSITIVITY

The minimum detectable concentration of BSA (Recombinant Bovine Serum Albumin) is 0.3826 ng/mL. The minimum detectable concentration was determined by adding twice standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

PRECISION

1. Intra-assay Precision

Three samples of known concentration were tested ten times on one plate to assess intra-assay precision , Intra-Assay Precision CV≤15%.

2. Inter-assay Precision

Three samples of known concentration were tested in three separate assays to assess inter-assay precision , Inter-Assay Precision CV≤15%.

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	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	10	10	10	3	3	3
Mean (ng/mL)	36.885	4.693	0.481	39.155	4.812	0.496
SD	1.786	0.222	0.043	2.268	0.154	0.023
CV (%)	4.8	4.7	8.9	5.8	3.2	4.7

Note: *The example data is for reference only.*

ACCUEACY

Five samples of known concentration were tested ten times on one plate to assess Accuracy, Accuracy recovery rate 80-120%.

Sample	BSA (Recombinant Bovine Serum Albumin)				
Sample Conc.(ng/mL)	40.5	30	5	1.2	0.5
n	10	10	10	10	10
Mean (ng/mL)	39.16	29.01	4.76	1.11	0.48
SD	1.682871566	1.773271568	0.148459014	0.103718394	0.029793978
CV (%)	4	6	3	9	6
Recovery（%）	97	97	95	92	97

Note: *The example data is for reference only.*

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LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of BSA were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture medium (DMEM)	Cell culture medium (MEM)	Serum
1:2	Average Recovery (%)	97.4	94.2	116.2
	Range (%)	89.6-111.8	94.5-104.1	110.8-123.9
1:4	Average Recovery (%)	98.4	102.3	114.7
	Range (%)	93.0-104.0	93.1-123.0	112.8-117.3
1:8	Average Recovery (%)	96.6	103.9	115.4
	Range (%)	93.4-103.6	96.9-108.5	109.6-120.4
1:16	Average Recovery (%)	96.0	104.3	113.2
	Range (%)	92.8-99.9	93.4-112.4	111.9-115.1

***Note**: The example data is for reference only.*

SPECIFICITY

Specificity-1: High, medium and low concentrations of BSA were added to Human serum (v/v 1%), Mouse serum (v/v 2.5%), Human Serum Albumin (1.25mg/mL) and Rabbit serum (v/v 10%), the recovery rate of BSA was used as the specific validation index. Recovery rate 80-125%.

Sample	1% Human serum			2.5% Mouse serum			Human Serum Albumin (1.25 mg/mL)			10% Rabbit serum		
Added BSA Conc.(ng/mL)	30	5	0	30	5	0	30	5	0	30	5	0
Mean (ng/mL)	25.805	4.239	0.0	24.319	4.369	0.327	26.009	4.535	0.056	35.252	5.995	0.3
Recovery （%）	86.0	85.9	/	80	87.4	/	86.5	89.6	/	107.4	119.3	/

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Specificity-2: High, medium and low concentrations of BSA were added to MDCK Cell, HEK293 Cell, CHO Cell, T Cell and VERO Cell, the recovery rate of BSA was used as the specific validation index. Recovery rate 80%-125%.

Sample	MDCK			HEK293			CHO		
Cells. Con.（cells/mL）	2×10^6			3.5×10^6			4.32×10^6		
Dilution Factor	2			2			2		
Added BSA Conc.(ng/mL)	30	5	0	30	5	0	30	5	0
Mean (ng/mL)	32.519	4.778	0	31.566	6.622	0.447	28.970	4.836	0.196
Recovery（%）	108.4	95.6	/	103.7	123.5	/	95.9	92.8	/

Sample	T-lymphocyte			Vero		
Cells. Con.（cells/mL）	2.15×10^6			3×10^6		
Dilution Factor	2			2		
Added BSA Conc.(ng/mL)	30	5	0	30	5	0
Mean (ng/mL)	31.509	4.862	0	28.502	4.306	0
Recovery（%）	105.0	97.2	/	95.0	86.1	/

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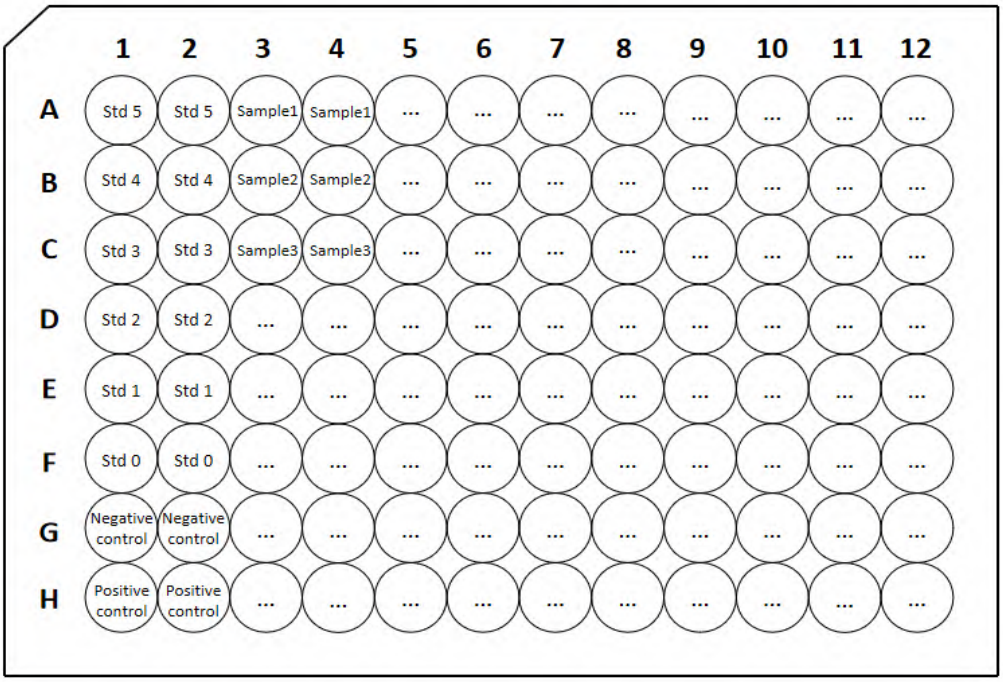
MATRIX INTERFERENCE

Verify potential matrix effects by adding different levels of specific buffers to the sample buffer. For specific buffers, it is recommended that you verify recovery to determine the optimal dilution ratio.

Additive	Tolerated concentration
1×PBS	100%
50 mM Tris	100%
DMSO	5%
Multiple Electrolytes Injection	100%
Glucose	10%
Dextran	10%
Fish gelatin	1%
DMEM	100%

LATE LAYOUT



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TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of theassay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts

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