Version 2c Last updated 21 April 2026

ab273188
Bovine Insulin ELISA Kit

For the quantitative measurement of Bovine Insulin in cell culture supernatants, plasma and serum.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Bovine Insulin ELISA Kit (ab273188) is an *in-vitro* enzyme-linked immunosorbent assay for the quantitative measurement of Bovine Insulin in serum (Bovine Insulin concentration is low in normal serum/plasma, and may not be detectable in this assay), plasma and cell culture supernatants.

This assay employs an antibody specific for Bovine Insulin coated on a 96-well plate. Standards and samples are pipetted into the wells and Insulin present in a sample is bound to the wells by the immobilized antibody. The wells are washed, and biotinylated anti- Bovine Insulin antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Insulin bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed.

Add 100 µL standard or sample to each well.
Incubate 2.5 hours at room temperature.

Add 100 µL prepared biotin antibody to each well.
Incubate 1 hour at room temperature.

Add 100 µL prepared Streptavidin solution.
Incubate 45 minutes at room temperature.

Add 100 µL TMB Substrate Reagent to each well.
Incubate 30 minutes at room temperature.

Add 50 µL Stop Solution to each well.
Read at 450 nm immediately.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All ELISA kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

4. Storage and Stability

The entire ELISA kit may be stored at -20°C.

Observe the storage conditions for individual prepared components in the Reagent Preparation section 9.

5. Limitations

- ELISA kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
500X HRP-Streptavidin Concentrate	200 µL	-20°C
20X Wash Buffer	25 mL	-20°C
5X Assay Diluent B	15 mL	-20°C
Assay Diluent C	30 mL	-20°C
Stop Solution	8 mL	-20°C
TMB Substrate Solution	12 mL	-20°C
Biotinylated Anti-Bovine Insulin Antibody	2 vials	-20°C
Anti-Bovine Insulin Coated Microplate (12 x 8 wells)	1 unit	-20°C
Bovine Insulin Standard (Lyophilized)	2 vials	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution or sample dilutions.
- 100 mL and 1 L graduated cylinders.

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 5X Assay Diluent B:

Dilute 5X Assay Diluent B 5-fold with deionized or distilled water before use.

9.2 Biotinylated Anti-Bovine Insulin Detection Antibody:

Add 100 µL of 1X Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B.

9.3 20X Wash Buffer:

If the Wash Concentrate (20X) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.

9.4 HRP-Streptavidin Concentrate:

Briefly spin the vial of HRP-Streptavidin concentrate before use. HRP-Streptavidin should be diluted 500-fold with 1X Assay Diluent B.

For example: Briefly spin the vial and pipette up and down to mix gently. Add 20 µL of HRP-Streptavidin concentrate into a tube with 10 mL 1X Assay Diluent B to prepare a 500-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Briefly spin the Standard Vial.
10.2 Add 400 µL of Assay Diluent C into Standard Vial to prepare a 1,400 µIU/mL Standard stock solution. Gently mix the powder to allow it to dissolve thoroughly.
10.3 Add 120 µl of Standard stock solution into a tube with 440 µl Assay Diluent C to prepare a 300 µIU/mL standard solution.
10.4 Pipette 250 µL Assay Diluent C into each tube.
10.5 Use the 300 µIU/mL Standard solution to produce a dilution series. Adding 250 µL from #1 to #2, then from #2 to #3, etc.
10.6 Mix each tube thoroughly before the next transfer.
10.7 Tube #8 contains no protein and is the Blank control.

Tube #	Volume to dilute	Volume of Assay Diluent C	Final Concentration µLU/mL
1	300 µIU/mL Standard Stock Solution	---	300
2	250 µL of tube #1	250 µL	150
3	250 µL of tube #2	250 µL	75
4	250 µL of tube #3	250 µL	37.5
5	250 µL of tube #4	250 µL	18.75
6	250 µL of tube #5	250 µL	9.38
7	250 µL of tube #6	250 µL	4.69
8	---	250 µL	0

11. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

Δ Note: Assay Diluent C should be used for dilution of serum, plasma, and cell culture supernatant samples. The suggested dilution for normal serum/plasma is 2-fold.

11.1 Label removable 8-well strips as appropriate for your experiment.

11.2 Add 100 µL of standard or sample into appropriate wells. Cover the wells and incubate for 2.5 hours at room temperature with gentle shaking.

11.3 Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel Pipette or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

11.4 Add 100 µL of 1X prepared biotinylated antibody to each well. Incubate for 1 hour at room temperature with gentle shaking.

11.5 Discard the solution. Repeat the wash as in step 11.3.

11.6 Add 100 µL of prepared Streptavidin solution each well. Incubate for 45 minutes at room temperature with gentle shaking.

11.7 Discard the solution. Repeat the wash as in step 11.3.

11.8 Add 100 µL of TMB Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

11.9 Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

12. Calculations

12.1 Calculate the mean absorbance for each set of duplicate standards, controls and samples.
12.2 Subtract the average zero standard optical density.
12.3 Plot the standard curve on log-log, with standard concentration on the x-axis and absorbance on the y-axis.
12.4 Draw the best-fit straight line through the standard points.

13. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

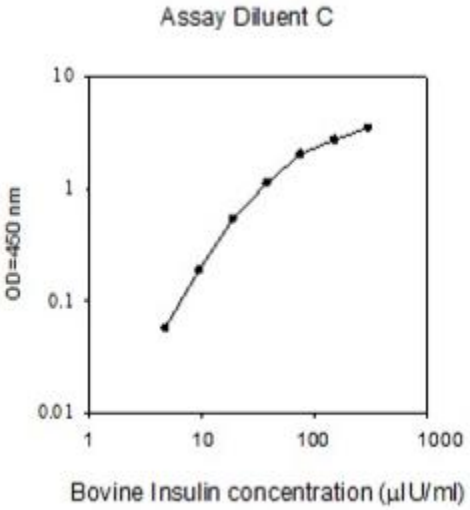

Figure 1. Bovine Insulin ELISA kit (ab273188) Standard curve.

Sensitivity:

The minimum detectable dose of Bovine Insulin was determined to be 4.5 µIU/mL.

Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

Reproducibility:

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%

Spiking and Recovery:

Recovery was determined by spiking various levels of Bovine insulin into the sample types listed below. Mean recoveries are as follows:

Sample Type	Average % recovery	Range %
Serum	90.59	72-114
Plasma	101.9	81-122
Cell culture media	113.1	87-140

Linearity:

Sample Type		Serum	Plasma	Cell culture media
1:2	Average % of expected	128.4	115.3	110.9
	Range %	114-143	96-137	104-118
1:4	Average % of expected	91.92	87.22	95.47
	Range %	81-103	75-100	87-104

Specificity:

This ELISA kit detects bovine, human, mouse, rat and porcine Insulin.

14. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Improper preparation of standard and/or biotinylated antibody	Briefly spin down vials before opening. Dissolve the powder thoroughly.
	Too brief incubation times	Ensure sufficient incubation time. Sample and standard addition may be done overnight at 4°C with gentle shaking (note: may increase overall signals including background).
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
	Air bubbles in wells	Remove bubbles in wells
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your standard at <-70°C after reconstitution, others at 4°C. Keep substrate solution protected from light.
	Stop solution	Add stop solution to each well before reading plate

15. Notes

Technical Support

For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)