# Cow RBBP4 ELISA Kit

**Catalog No.:** abx380447

**Size:** 96T

**Range:** 78.1 pg/ml - 5000 pg/ml

**Sensitivity:** < 46.9 pg/ml

**Storage:** Store at 4°C for up to 6 months.

**Application:** For quantitative detection of RBBP4 in Cow Serum, Plasma, Tissue Homogenates and other biological fluids.

**Introduction:** Core histone-binding subunit that may target chromatin assembly factors, chromatin remodeling factors and histone deacetylases to their histone substrates in a manner that is regulated by nucleosomal DNA. Component of several complexes which regulate chromatin metabolism. These include the chromatin assembly factor 1 (CAF-1) complex, which is required for chromatin assembly following DNA replication and DNA repair; the core histone deacetylase (HDAC) complex, which promotes histone deacetylation and consequent transcriptional repression; the nucleosome remodeling and histone deacetylase complex (the NuRD complex), which promotes transcriptional repression by histone deacetylation and nucleosome remodeling; the PRC2/EED-EZH2 complex, which promotes repression of homeotic genes during development; and the NURF (nucleosome remodeling factor) complex.

**Principle of the Assay**

This kit is based on sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific to RBBP4 is pre-coated onto a 96-well plate. The standards and test samples are added to the wells and washed with wash buffer. Biotin conjugated antibody specific to RBBP4 is used as a detection antibody. TMB substrate is used to visualize HRP activity. TMB is catalyzed by HRP to produce a blue colour product that changes into yellow after adding stop solution. The intensity of the color yellow is proportional to the RBBP4 amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of RBBP4 can be calculated.

**Kit components**

1. One pre-coated 96 well plate
2. Standard: 2 tubes
3. Sample/Standard Diluent Buffer: 20 ml
4. Biotin conjugated antibody (Dilution 1:100): 120 µl
5. Antibody diluent buffer: 10 ml
6. Streptavidin-HRP conjugate (SABC) (Dilution 1:100): 120 µl
7. SABC diluent buffer: 10 ml
8. TMB substrate: 10 ml
9. Stop solution: 10 ml
10. Wash buffer (25X): 30 ml
11. Plate Sealer: 5

**Material Required But Not Provided**

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. Precision pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5 ml tubes to prepare standard/sample dilutions
7. Absorbent filter papers
8. 100 ml and 1 L volume graduated cylinders

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## Protocol

### A. Preparation of sample and reagents

#### 1. Sample

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.
- **Tissue homogenates:** The preparation of tissue homogenates will vary depending upon tissue type – this is just an example. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 5000 × g for 5 min and collect the supernatant. Assay immediately or aliquot and store at -20°C.
- **Other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

**Note:**

» Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.

» Samples should be clear and transparent. Samples must be diluted so that the expected concentration falls within the kit's range.

» Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used. Samples that contain $NaN_3$ cannot be detected as it interferes with HRP.

» Always use non-pyrogenic, endotoxin-free tubes for blood collection.

**Sample dilution guideline:**

Estimate the concentration of the target in the sample and select the correct dilution factor to make the diluted target concentration fall near the middle of the kit's range. Generally, for high concentration (50000 pg/ml - 500000 pg/ml), dilute 1:100, for medium concentration (5000 pg/ml - 50000 pg/ml), dilute 1:10 and for low concentration (78.1 pg/ml - 5000 pg/ml), dilute 1:2. Very low concentrations (≤ 78.1 pg/ml ) do not need dilution. Dilute the sample with the provided Sample Diluent Buffer and mix thoroughly. Several trials may be necessary to determine the optimal dilution factor.

#### 2. Wash buffer

Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 30 ml of concentrated wash buffer into 720 ml of distilled water).

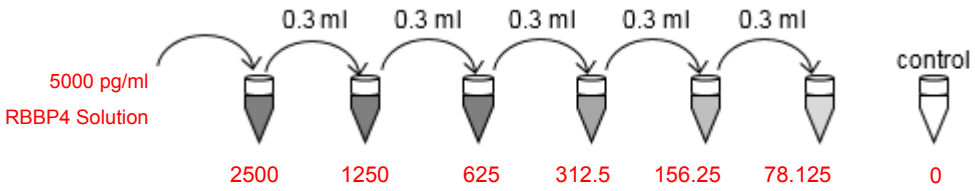
#### 3. Standard

Preparation of the RBBP4 standard: standard solution should be prepared no more than 15 min prior to the experiment. Centrifuge at 10,000×g for 1 minute as the powder may drop off from the cap when opening if you do not spin down. **(Note: Do not dilute the standard directly in the plate).**

a.) 5000 pg/ml standard solution. Add 1 ml of Sample/Standard diluent buffer into one Standard tube. Allow the reconstituted standard to sit for 15 minutes with gentle agitation prior to carrying out the serial dilutions; avoiding foaming or bubbles.

b.) 2500 pg/ml → 78.125 pg/ml standard solutions: Label 6 tubes with 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 312.5 pg/ml, 156.25 pg/ml and 78.125 pg/ml. Aliquot 0.3 ml of the Sample / Standard diluent buffer into each tube. Add 0.3 ml of the above 5000 pg/ml standard solution into 1st tube and mix thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix thoroughly, and so on.

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**Note:** Do not vortex the standard during reconstitution, as this will destabilize the protein. Once your standard has been reconstituted, it should be used right away. We do not recommend reusing the reconstituted standard. Please use the diluted Standards for a single assay procedure and discard after use.

**4. Preparation of Biotin conjugated antibody working solution:** prepare no more than 1 hour before the experiment.
a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).
b.) Dilute the Biotin conjugated antibody with antibody diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of Biotin conjugated antibody into 99 µl of antibody diluent buffer.

**5. Preparation of Streptavidin-HRP Conjugate (SABC) working solution:** prepare no more than 30 min. before the experiment
a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).
b.) Dilute the SABC with SABC diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of SABC into 99 µl of SABC diluent buffer.

**B. Assay Procedure**
Equilibrate the SABC working solution to room temperature and TMB substrate at 37°C for 30 minutes prior to use. It is recommended to plot a standard curve for each test.

1. **Wash the plate two times before adding standard, samples and buffers.** Any strips that are not being used should be kept dry and stored at 4°C. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate.
2. Add 100 µl of the prepared standards solutions into the standard wells.
3. Add 100 µl of Sample / Standard diluent buffer into the control (zero) well.
4. Add 100 µl of appropriately diluted sample into test sample wells.
5. Cover the plate and incubate at 37°C for 90 minutes.
6. Remove the cover, discard the liquid and wash the plate two times with wash buffer.
7. Add 100 µl of prepared Biotin conjugated antibody working solution into each well (standard, test sample and zero well). Add the solution at the bottom of each well without touching the side walls. Seal the plate with a cover and incubate at 37°C for 60 minutes.
8. Remove the cover and wash the plate 3 times. Discard the solution without touching the side walls. Blot the plate on an absorbent material. Fill each well completely with wash buffer and soak for at least 1-2 min. Invert the plate each time and decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid. Repeat this procedure for a total of three times.

**Please note:** For automated washing, discard the solution in all wells and wash three times overfilling the wells with Wash buffer. After the final wash invert the plate and tap on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min.

9. Add 100 µl of SABC working solution into each well, cover the plate and incubate at 37°C for 30 minutes.

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10. Remove the cover and wash the plate 5 times with Wash buffer. Allow the wash buffer to remain in the wells 1-2 min for each wash. Discard the washing buffer and blot the plate onto absorbent filter papers or other absorbent material.
11. Add 90 µl of TMB substrate into each well. Cover the plate and incubate at 37°C in dark conditions for 15-20 minutes (incubation time is for reference only, do not exceed 30 minutes). When an apparent gradient appears in the standard wells the reaction can be terminated.
12. Add 50 µl of Stop solution into each well (including the blank well). There should be a color change to yellow. Gently tap the plate to ensure thorough mixing.
13. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). Log-log curve fitting is recommended for data analysis. The Cow RBBP4 concentration of the samples can be interpolated from the standard curve.

**Note:** If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

**C. Precautions**

1. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.
2. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
3. Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.
4. Avoid foaming or bubbles when mixing or reconstituting components. Prepare the Standard solutions within 15 min of starting the experiment. Please use the diluted Standard for a single assay procedure and discard after use.
5. It is recommended to assay all standards, controls and samples in duplicate or triplicate.
6. Do not let the plate dry out completely during the assay as this will inactivate the biological material on the plate.
7. Ensure plates are properly sealed or covered during incubation steps.
8. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
9. To avoid cross contamination do not reuse pipette tips and tubes.
10. Do not use components from a different kit or expired ones.
11. The TMB Substrate solution is easily contaminated; work under sterile conditions when handling the TMB substrate solution. The TMB Substrate solution should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

**D. Precision**

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of RBBP4 were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of RBBP4 were tested on 3 different plates, 8 replicates in each plate.

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CV (%) = (Standard Deviation / mean) × 100

Intra-Assay: CV<8%

Inter-Assay: CV<10%

**Sample Recovery (after spiking)**

| Sample Type | Range | Average Recovery |
|---|---|---|
| Serum (n=5) | 88-105% | 98% |
| EDTA Plasma (n=5) | 93-103% | 97% |
| Heparin Plasma (n=5) | 88-103% | 95% |

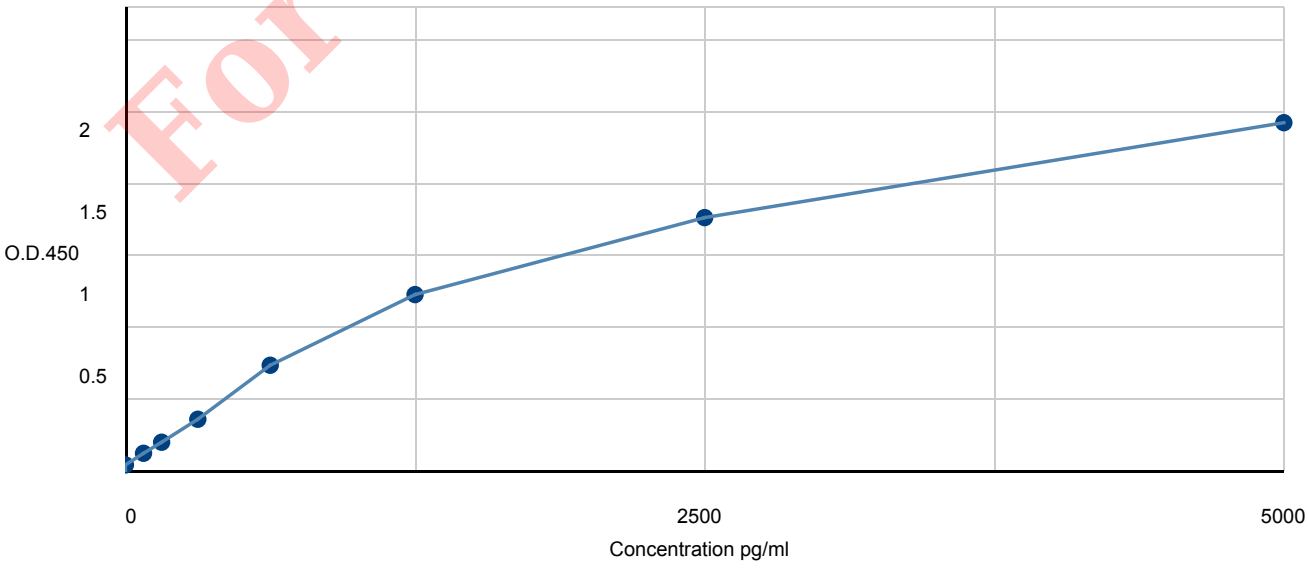
**Linearity**

| Sample Type | 1:2 | 1:4 | 1:8 | 1:16 |
|---|---|---|---|---|
| Serum (n=5) | 92-102% | 85-99% | 86-102% | 88-100% |
| EDTA Plasma (n=5) | 87-99% | 82-101% | 84-101% | 90-102% |
| Heparin Plasma (n=5) | 82-93% | 83-100% | 83-97% | 85-98% |

**E. Typical Data & Standard Curve**

Typical Standard Curve Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

| **Concentration pg/ml** | 0 | 78.125 | 156.25 | 312.5 | 625 | 1250 | 2500 | 5000 |
|---|---|---|---|---|---|---|---|---|
| **OD450** | 0.037 | 0.118 | 0.195 | 0.355 | 0.731 | 1.223 | 1.759 | 2.42 |




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