Rat HMGB-1(High Mobility Group Protein B1) ELISA Kit
Catalog NO.: abk9503R

This ELISA kit applies to the in vitro quantitative determination of Rat HMGB-1 concentrations in serum, plasma and other biological fluids. This manual must be read attentively and completely before using this product. If you have any problems, please contact our Technical Service Center for help.

Phone: +91 12041 39569
Email: info@nulifecare.in
Website: www.nulifecare.in

Please refer to specific expiry date on the label outside of box.

Please kindly provide us with the lot number (on the outside of the box) of the kit for more efficient service.

Character

Sensitivity	18.75 pg/mL
Detection Range	31.25-2000pg/mL
Specificity	This kit recognizes Rat HMGB-1 in samples. No significant cross-reactivity or interference between Rat HMGB-1 and analogs was observed.
Repeatability	Coefficient of variation is ≤ 10%

Test principle

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Rat HMGB-1. Samples (or Standards) are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Rat HMGB-1 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Rat HMGB-1, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of Rat HMGB-1. You can calculate the concentration of Rat HMGB-1 in the samples by comparing the OD of the samples to the standard curve.

Note for kit

1. Please wear lab coats, goggles and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories, especially when detecting blood samples or other bodily fluids.
2. A freshly opened ELISA plate may appear a water like substance, which is normal and will not have any impact on the experimental results. Return the unused wells to the foil pouch and store according to the conditions suggested in the above table.
3. Do not reuse the reconstituted standard, biotinylated detection Ab working solution, concentrated HRP conjugate working solution. The unspent undiluted concentrated biotinylated detection Ab (100×) and other stock solutions should be stored according to the storage conditions in the table.
4. Do not use expired reagents. If using the kit in multiple sessions, prepare each component according to the required amount to avoid waste and ensure proper use in subsequent experiments. It is recommended to keep the microplate frame, return any unused wells to the aluminum foil bag, and ensure that all components are used within their shelf life.
5. Do not mix or substitute reagents from other batches or sources with those provided in this kit.
6. Do not reuse diluted standards, biotinylated antibody working solutions, or enzyme conjugate working solutions. The highest concentration standard working solution can be stored at -20°C for up to two weeks, but should not be freeze-thawed repeatedly.
7. It is recommended that all samples and standards be assayed in duplicate.

Kit components & Storage

An unopened kit can be stored at 2-8℃ for 6 months. If the opened kit is not used up, store the items separately according to the following conditions.

Item	Specifications	Storage
Micro ELISA Plate	96T: 8 wells×12 strips 48T: 8 wells×6 strips 24T: 8 wells×3 strips	-20℃, 6 months
Reference Standard	96T: 2 vials 48T: 1 vial 24T: 1 vial	
Concentrated Biotinylated Detection Ab (100X)	96T:1 vial, 120μL 48T:1 vial, 60μL 24T:1 vial, 60μL	
Concentrated HRP Conjugate (100X)	96T:1 vial, 120μL 48T:1 vial, 60μL 24T:1 vial, 60μL	-20℃, 6 months
Reference Standard & Sample Diluent	1 vial, 20 mL	-20℃, 6 months
Biotinylated Detection Ab Diluent	1 vial, 13 mL	
HRP Conjugate Diluent	1 vial, 13 mL	
Concentrated Wash Buffer (25X)	1 vial, 30 mL	
Substrate Reagent	1 vial, 10 mL	2-8℃ (Protect from light)
Stop Solution	1 vial, 10 mL	-20℃, 6 months
Plate Sealer	5 pieces	RT
Product Description	1 copy	
Certificate of Analysis	1 copy	

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution. The volume of reagents in partial shipments is a little more than the volume marked on the label, please use accurate measuring equipment instead of directly pouring into the vial(s).

Other supplies required

1. Microplate reader with 450 nm wavelength filter(preheat it for 15 min before OD measurement)
2. High-precision transfer pipette, EP tubes and disposable pipette tips
3. Incubator capable of maintaining 37℃
4. Deionized or distilled water
5. Absorbent paper
6. Loading slot

Sample collection

1. **Serum:** Allow samples to clot for 1 hour at room temperature or overnight at 2-8℃ before centrifugation for 20 min at 1000×g at 2-8℃. Collect the supernatant to carry out the assay.
2. **Plasma:** Collect plasma using EDTA-Na_2 as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2-8℃ within 30 min of collection. Collect the supernatant to carry out the assay.
3. **Cell culture supernatant:** Centrifuge samples for 20 min at 1000×g at 2-8℃. Collect the supernatant to carry out the assay.

Note for sample

① The blood collection tubes should be single-use, pyrogen-free, and endotoxin-free. Whole blood samples should not be directly frozen; they must be processed into serum or plasma for testing or storage.

② For ELISA experiments, common anticoagulants such as sodium and potassium EDTA, and heparin salts can be used to collect plasma samples. However, for other experiments, such as biochemical assays, the researcher should assess the impact of the anticoagulants.

③ Samples should be assayed within 7 days when stored at 2-8℃, otherwise samples must be divided up and stored at -20℃ (≤1 month) or -80℃ (≤3 months). Avoid repeated freeze thaw cycles. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates.

④ Do not use lysis buffers containing surfactants (such as SDS) or organic reagents (such as methanol) to prepare tissue homogenates, cell lysates, or other types of samples.

⑤ Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.

Sample Testing Protocol

1. Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.
2. We are only responsible for the kit itself, but not for the samples consumed during the assay. The users should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
3. The detection range of the kit is not equivalent to the concentration range of the analyte in the sample. It is recommended to consult reference, conduct preliminary experiments, or seek technical support to estimate the concentration of the analyte in your sample. If the analyte concentration in the sample is too high or too low, appropriate dilution or concentration of the sample should be performed.
4. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
5. If your sample requires dilution and the dilution factor is high, it is recommended to refer to the general dilution guidelines.

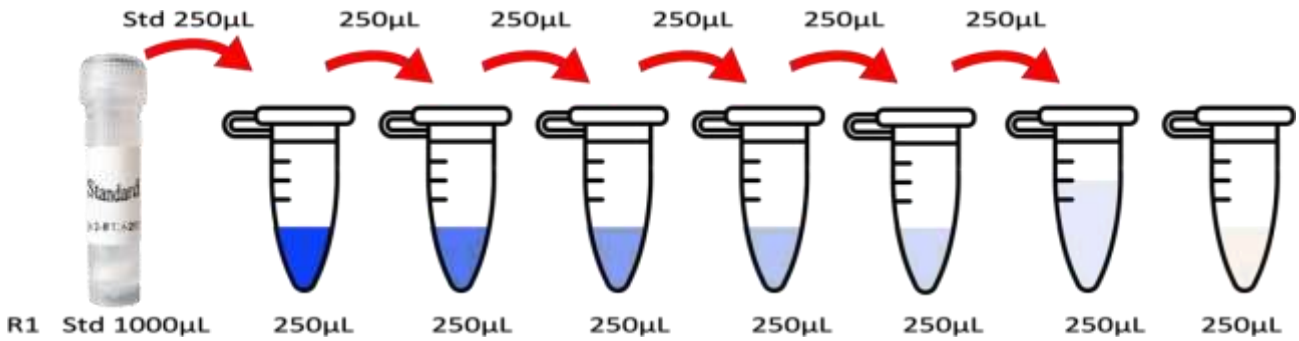
Reagent preparation

1. Bring all reagents to room temperature (18-25℃) before If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment, and store the remaining strips and reagents at required condition.
2. Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40 ℃ water bath and mix it gently until the crystals have completely dissolved.
3. Standard working solution:

① **Centrifuge:** Centrifuge the standard at 10, 000×g for 1 min.

② Add 1mL of Reference Standard &Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 2000pg/mL.

③ Serial dilution: Take 7 EP tubes, adding 250μL of reference standard & sample dilution buffer to each tube (The volume can be adjusted based on actual usage, e.g., 500μL/ tube). Transfer 250μL of the 2000pg/mL standard working solution into the first tube and mix thoroughly to obtain the 1000pg/mL standard working solution. Continue the dilution step by step until the second-to-last tube. The last tube will serve as the blank, and no solution should be transferred from the second-to-last tube. The standard working solution should be freshly prepared and used immediately.



2000	1000	500	250	125	62.5	31.25	0
pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL

4. Biotinylated Detection Ab working solution: Calculate the required amount before the experiment (100μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated Biotinylated Detection Ab at 800×g for 1 min, then dilute the 100× Concentrated Biotinylated Detection Ab to 1× working solution with Biotinylated Detection Ab Diluent (Concentrated Biotinylated Detection Ab: Biotinylated Detection Ab Diluent= 1: 99).

5. HRP Conjugate working solution: Calculate the required amount before the experiment (100μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate at 800×g for 1 min, then dilute the 100× Concentrated HRP Conjugate to 1× working solution with HRP Conjugate Diluent (Concentrated HRP Conjugate: HRP Conjugate Diluent= 1: 99).

6. Experimental Operation Tips

① Solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.

② Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.

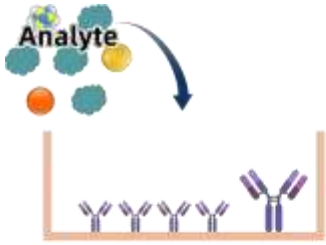
③ After adding the Substrate Reagent. The reaction time can be shortened or extended according to the actual color change, but not more than 30 min.

④ Adding the stop solution should be done in the same order as the substrate solution.

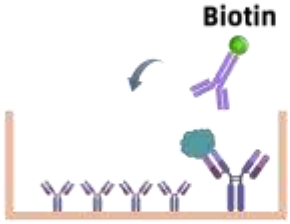
Assay procedure

1. Determine wells for diluted standard, blank and sample. Add 100µL each dilution of standard, blank and sample into the appropriate wells.
2. Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37°C. Decant the solution from each well and pat it dry against clean absorbent paper.
3. Add 300µL of wash buffer to each well. Soak for 0.5 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times.
4. Add 100µL of Biotinylated Detection Ab working solution to each well. Cover the plate with a new sealer. Incubate for 1 hour at 37°C.
5. Decant the solution from each well, repeat the wash process for 3 times as conducted in step 3.
6. Add 100µL of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 30 min at 37°C.
7. Decant the solution from each well, repeat the wash process for 3 times as conducted in step 3.
8. Add 100µL of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37°C. Protect the plate from light.
9. Add 50µL of Stop Solution to each well.
10. Determine the Optical Density of each well at once with a micro plate reader set to 450 nm.

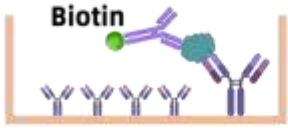
Assay Procedure Summary



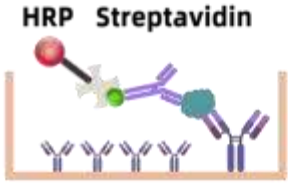
1、Add 100μL standard or sample to the wells. Incubate for 90 min at 37℃. Aspirate and wash plate for 3 times



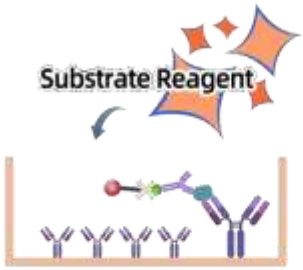
2、Immediately add 100μL Biotinylated Detecting Ab working solution to each well. Incubate for 60 min at 37℃



3、Aspirate and wash the plate for 3 times



4、Add 100μL HRP conjugate working solution. Incubate for 30 min at 37℃



5、Aspire and wash the plate for 3 times. Add 100μL Substrate Reagent. Incubate for 15 min at 37℃



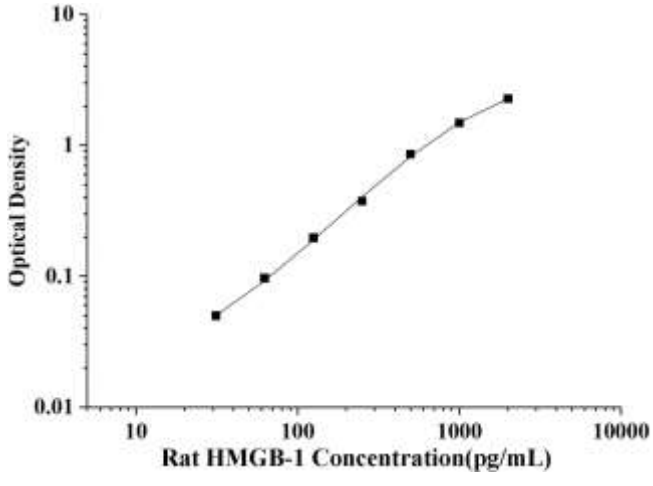
6、Add 50μL Stop Solution



7、Read the plate at 450nm immediately. Calculation of the results

Calculation of results

1. The reference standard curve in this manual is for guidance only. The experimenter should use the standard data from each experiment to construct the standard curve and calculate the concentration of the target substance in the sample.
2. When constructing the standard curve, first calculate the average OD values of the standards and sample replicates, and subtract the OD value of the blank well as the correction value. Then, plot the concentration on the X-axis and the corrected OD values on the Y-axis, and use software to perform a nonlinear four-parameter logistic (4PL) fit. This will provide the standard curve, from which the sample concentration can be calculated.
3. The actual concentration is the calculated concentration multiplied by the dilution factor.

pg/mL	Corrected OD	Standard Curve
2000	2.284	
1000	1.49	
500	0.859	
250	0.376	
125	0.197	
62.5	0.097	
31.25	0.05	
0	0	

Permanence

Specificity: This kit is designed for the detection of Rat HMGB-1. It has been tested and shows no significant cross-reactivity with other similar substances. However, due to technical limitations and the variability of sample sources, it is not possible to test all related or similar substances for cross-reactivity. Therefore, this kit may potentially exhibit cross-reactivity with other substances that have not been tested.

Sensitivity: The minimum detectable Rat HMGB-1 is 18.75 pg/mL.
Sensitivity is determined by calculating the average OD value of 20 blank wells plus 3 times the standard deviation, then converting it to the corresponding concentration.

Repeatability: The precision of this kit is ≤10%, meeting the precision quality control standard. Precision is divided into intra-plate precision and inter-plate precision. It is evaluated by the coefficient of variation (CV) of sample values measured using the same batch and different batches of the kit.
CV (%) = Standard Deviation (SD) / Mean × 100.

Recovery： The recovery rate of this kit is 80-120%, meeting the recovery rate quality control standard. The recovery rate is divided into spiked recovery and sample dilution linear recovery. Spiked Recovery: Known concentrations of Rat HMGB-1 are added to different samples for recovery experiments, resulting in the recovery rate range and average value. Sample Dilution Linear Recovery:Samples containing Rat HMGB-1 are diluted at different ratios (e.g., 2x, 4x, etc.), and the recovery rate range and average value are determined.

Stability: It has been determined that when stored according to the instructions, the kit performs best within its expiration date.

Declaration

1. Limited by current conditions and scientific technology, we can't conduct comprehensive identification and analysis of all the raw material provided. There might be some qualitative and technical risks for users using the kit.
2. This assay is designed to eliminate interference by factors present in biological samples. Until all factors have been tested in the ELISA immunoassay, the possibility of interference cannot be excluded.
3. The final experimental results will be closely related to the validity of products, operational skills of the operators, the experimental environments and so on.We are only responsible for the kit itself.
4. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions.
5. Incorrect results may occur because of incorrect operations during the reagents preparation and loading, as well as incorrect parameter settings of the Micro plate reader. Please read the instructions carefully and adjust the instrument prior to the experiment.
6. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
7. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some variables such as transportation conditions, different lab equipment, and so on. Intra assay variance among kits from different batches might arise from the above reasons too.
8. Kits from different manufacturers or other methods for testing the same analyte could bring out inconsistent results, since we haven’t compared our products with those from other manufacturers.
9. The kit is designed for research use only, we will not be responsible for any issues if the kit is applied in clinical diagnosis or any other related procedures.

Plate Layout

Use this plate layout to record standards and samples assayed.

