

ABIN7041531

# Sandwich ELISA Kit for Hemoglobin

Rat

96 tests

For research use only

Not for use in clinical diagnostic procedures

Version Oct 2024

## Intended use

The kit is a sandwich enzyme immunoassay for the in vitro quantitative measurement of hemoglobin in rat serum, plasma.

## Reagents and materials provided

- ELISA Micro Plate antibody coated
- Enzyme Conjugated Detection Antibody (100X)
- Calibrator
- Diluent Concentrate (5X)
- Wash Solution Concentrate (20X)
- Chromogen - Substrate Solution
- STOP Solution

## Materials required but not supplied

1. Microplate reader with  $450 \pm 10\text{nm}$  filter.
2. Precision single or multi-channel pipettes and disposable tips.
3. Microcentrifuge tubes for diluting samples.
4. Squirt bottle or Microtitre washer
5. Deionized or distilled water.
6. Container for Wash Solution
7. Centrifuge for sample collection
8. Anticoagulant for plasma collection
9. Incubator capable of maintaining  $37\text{ }^{\circ}\text{C}$ .
10. Microplate shaker

## Storage of the kit

1. For unopened kit: All reagents should be stored according to the labels on the vials. All reagents should be stored at  $4\text{ }^{\circ}\text{C}$  for long term storage. Keep away from heat or direct sunlight.

2. For opened kits: the remaining reagents must be stored according to the above storage conditions. In addition, please return the unused wells to the foil pouch containing the desiccant and seal the foil pouch with the zipper.

**Note:**

It is strongly recommended to use the remaining reagents within 1 month, if this is done before the expiry date of the kit. Please refer to the label on the kit packaging for the expiration date of the kit. All components are stable until the expiration date.

## Sample collection and storage

| Sample type | Collection procedure                                                                                                                                                                                                                                                                                                                      |
|-------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Plasma      | Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2-8°C within 30 min of collection. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles. Hemolysed samples are not suitable for ELISA assay! |
| Serum       | Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 min at 1000×g. Collect the supernatant and assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.                 |
| Urine       | Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge for 15 min at 1000×g at 2-8°C to remove particulate matter, assay immediately or aliquot and store at -20°C. Avoid repeated freeze-thaw cycles.                                                                         |

**Note:**

1. Samples to be used within 5 days may be stored at 4 °C, otherwise samples must be stored at -20 °C (≤ 1 month) or -80 °C (≤ 2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be used.
3. When performing the assay, bring samples to room temperature.

## Reagent preparation

- Bring all reagents to room temperature (16°C to 25°C) before use.
- Diluent Concentrate - The Diluent Solution supplied is a 5X Concentrate and must be diluted 1/5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH<sub>2</sub>O).
- Wash Solution Concentrate - The Wash Solution supplied is a 20X Concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH<sub>2</sub>O). Crystal formation in the concentrate may occur when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.
- Enzyme-Antibody Conjugate - Calculate the required amount of working conjugate solution for each microtitre plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1X Diluent for each test strip to be used for testing. Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.
- Pre-coated ELISA Micro Plate - Ready to use as supplied. Unseal foil pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.
- Calibrator – Prepare according to the lot specific Certificate of Analysis.

## Sample preparation

- It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturation may occur in these samples, leading to false results. Samples should therefore be stored for a short period at 2 - 8 °C or aliquoted at -20 °C ( $\leq 1$  month) or -80 °C ( $\leq 3$  months). Repeated freeze-thaw cycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates.
- If the sample type is not specified in the instructions, a preliminary test is necessary to determine compatibility with the kit.
- If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance. The recommended dilution factor is for reference only.
- Please estimate the concentration of the samples before performing the test. If the values are not in the range of the standard curve, the optimal sample dilution for the particular experiment has to be determined.

### Note:

The assay requires that each test sample be diluted before use. All samples should be

assayed in duplicate each time the assay is performed. The recommended dilutions are only suggestions. Dilutions should be based on the expected concentration of the unknown sample such that the diluted sample falls within the dynamic range of the standard curve. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

Serum samples – Recommended starting dilution is 1/10,000. To prepare a 1/10,000 dilution of a sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1/100 dilution. Next, dilute the 1/100 by transferring 5 µL into 495 µL of 1X diluent. This gives you a 1/10,000 dilution. Mix thoroughly each stage.

Plasma samples – Recommended starting dilution is 1/10,000. To prepare a 1/10,000 dilution of a sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1/100 dilution. Next, dilute the 1/100 by transferring 5 µL into 495 µL of 1X diluent. This gives you a 1/10,000 dilution. Mix thoroughly each stage.

## Assay procedure

1. All samples and standards should be assayed in duplicates.
2. The Standards and the test sample(s) should be loaded into the ELISA wells as quickly as possible to avoid a shift in OD readings. Using a multichannel pipette would reduce this occurrence. Pipette 100 µL of Standard 0 (0.0 ng/mL) in duplicate Standard 1 (3.13 ng/mL) in duplicate Standard 2 (6.25 ng/mL) in duplicate Standard 3 (12.5 ng/mL) in duplicate Standard 4 (25 ng/mL) in duplicate Standard 5 (50 ng/mL) in duplicate Standard 6 (100 ng/mL) in duplicate Standard 7 (200 ng/mL) in duplicate
3. Pipette 100 µL of sample (in duplicate) into pre designated wells.
4. Incubate the micro titer plate at room temperature for sixty ( $60 \pm 2$ ) minutes. Keep plate covered and level during incubation.
5. Following incubation, aspirate the contents of the wells.
6. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.
7. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for thirty ( $30 \pm 2$ ) minutes. Keep plate covered in the dark and level during incubation.
8. Wash and blot the wells as described in Steps 5/6.
9. Pipette 100 µL of TMB Substrate Solution into each well.
10. Incubate in the dark at room temperature for precisely ten (10) minutes.
11. After ten minutes, add 100 µL of Stop Solution to each well.
12. Determine the absorbance (450 nm) of the contents of each well within 30 minutes. Calibrate the plate reader to manufacturer's specifications.

## Calculation of results

1. Subtract the average background value (Average absorbance reading of Standard zero) from the test values for each sample.
2. Average the duplicate readings for each standard and use the results to construct a Standard Curve. Construct the standard curve by reducing the data using computer software capable of generating a four parameter logistic curve fit. A second order polynomial (quadratic) or other curve fits may also be used; however, they will be a less precise fit of the data.
3. Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the target concentration in original samples.

## Typical data

In order to make the calculation easier, we plot the O.D. value of the standard (X-axis) against the log of concentration of the standard (Y-axis), although concentration is the independent variable and O.D. value is the dependent variable. The O.D. values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects).

## Detection range

The detection range of the kit is 3.13 ng/mL - 200 ng/mL

The standard curve concentrations used for the ELISA's were 200ng/mL, 100ng/mL, 50ng/mL, 25ng/mL, 12.5ng/mL, 6.25ng/mL, 3.13ng/mL, 0.0ng/mL

## Stability

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 5 % prior to the expiration date under appropriate storage condition. To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly monitored. It is also strongly suggested that the assay is performed by the same operator from the beginning to the end.

## Assay procedure summary

In this assay the hemoglobin (HM) present in samples reacts with the anti-HM antibodies, which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, anti-HM antibodies conjugated with horseradish peroxidase (HRP) are added. These enzyme-labeled antibodies form complexes with the

previously bound HM. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of HM in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of HM in the test sample. The quantity of HM in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

## Important note

1. The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.
2. Limited by the current conditions and scientific technology, we cannot perform a complete identification and analysis of the raw material used. Therefore, the use of the kit may be associated with some qualitative and technical risks.
3. We are only responsible for the kit itself, not for the samples used in the test. The possible amount of sample used in the whole test should be calculated in advance and sufficient sample material should be provided.
4. Each kit undergoes a very strict QC testing. Nevertheless, end-user results may differ from our internal results due to unexpected transport conditions or different laboratory equipment. Intra-assay deviations between kits from different lots can also be related to this.
5. The test results depend on the validity of the products, so the kit should be used before the expiration date and stored according to the instructions.
6. Even the same user may obtain different results in two separate experiments. To obtain reproducible results, each step of the assay should be controlled.
7. The standard of the kit and immunogen used for antibody preparation are commonly recombinant proteins, as different fragments, expression systems, purification methods might be used in recombinant protein preparation, we can not guarantee the kit could detect recombinant protein from other companies. So, it is not recommended to use the kit for the detection of recombinant protein.
8. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by the manufacturer.
9. Protect all reagents from strong light during storage and incubation. All reagent bottle caps should be tightly closed to prevent evaporation and contamination with microorganisms. The TMB substrate should remain colourless until it reacts with the enzyme.
10. A freshly opened ELISA plate may show a water-like substance, which is normal and does not affect the test results. Return unused wells to the foil pouch and store as

before.

11. Mistakes in reagent preparation and application, as well as incorrect parameter setting for the plate reader, can lead to incorrect results. A microplate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. at a wavelength of  $450 \pm 10$  nm is suitable for absorbance measurement. Please read the instructions carefully and set up the instrument before the experiment.
12. Do not reuse the reconstituted standard and the prepared working solutions. The unused stock solutions should be stored according to the storage conditions.

## Precaution

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

## Troubleshooting

| Problem             | Possible Source                             | Corrective Action                                       |
|---------------------|---------------------------------------------|---------------------------------------------------------|
| Poor Standard Curve | Improper standard curve preparation         | Ensure accurate operation of the dilution               |
|                     | Incomplete washing and aspiration           | Adequate washing and adequate aspiration                |
|                     | Inaccurate Pipetting                        | Check and Calibrate pipettes                            |
| Poor Precision      | Incomplete washing of wells                 | Ensure sufficient washing                               |
|                     | Inadequate mixing and aspiration reagents   | Adequate aspiration and mixing reagents                 |
|                     | Reused pipette tips, containers and sealers | Change and use new pipette tips, containers and sealers |
|                     | Inaccurate Pipetting                        | Check and Calibrate pipettes                            |
| Low O.D Values      | Inadequate reagent volumes added to wells   | Calibrate pipettes and add adequate reagents            |
|                     | Incorrect incubation times                  | Ensure sufficient incubation times                      |
|                     | Incorrect incubation temperature            | Reagents balanced to room temperature                   |

| Problem       | Possible Source                            | Corrective Action                                             |
|---------------|--------------------------------------------|---------------------------------------------------------------|
|               | Conjugate or substrate reagent failure     | Mix conjugate and substrate, color should develop immediately |
|               | No stop solution added                     | Follow the assay protocol in the kit manual                   |
|               | Read beyond suggested reading time         | Read within the time recommended in the manual                |
| Sample Values | Improper Sample Storage                    | Store the sample properly and use the fresh sample            |
|               | Improper sample collection and preparation | Take proper sample collection and preparation method          |
|               | Low quantity of analyte in samples         | Resample and repeat assay                                     |

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