Version 26-11/24

User's Manual

Human TDP43(Tar DNA Binding Protein 43KDa) ELISA Kit

REF **DEIA8708**

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RUO

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

In vitro quantitative determination of TDP43 concentrations in Serum, plasma, cell culture supernatant, cell lysate or tissue lysate, other biological fluid samples.

General Description

Tar DNA Binding Protein 43KDa (TDP-43) is a nuclear RNA-binding protein involved in RNA processing, splicing, and transport. It regulates the expression of target genes by binding to RNA transcripts and modulating their stability, localization, and translation. TDP-43 is predominantly localized in the nucleus under normal conditions, but it can shuttle between the nucleus and cytoplasm in response to cellular stress or pathological stimuli. Dysregulation of TDP-43 expression or localization is associated with neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD), where it forms pathological aggregates and disrupts RNA metabolism.

Principles of Testing

This kit was based on sandwich ELISA method. The experiment lasted 120 minutes. Capture antibody was conjugated to an affinity tag that was recognized by a specific antibody coated on the plate. Add the Cap/Det Ab working solution into each well, then add the standards and pilot samples into individual wells. If the sample contains TDP43, a capture antibody-TDP43-biotin-detection antibody complex was formed. After incubation, unbound conjugates were removed by wash buffer. HRP-Streptavidin was added. After washing, TMB substrates were added to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that turned yellow after adding a stop solution. Read the O.D. absorbance at 450 nm in a microplate reader. The concentration of TDP43 in the sample was calculated by drawing a standard curve. The concentration of the target substance is proportional to the OD450 value.

Reagents And Materials Provided

1. ELISA Microplate(Dismountable), 8×12. Put the rest strips into a sealed foil bag with the desiccant. Stored for 1 month at 2-8°C; Stored for 6 month at -20°C.
2. Lyophilized Standard, 2 vials. Put the rest standards into a desiccant bag. Stored for 1 month at 2-8°C; Stored for 6 month at -20°C.
3. Cap/Det Ab (Ready to use), 6 ml. 2-8°C (Avoid Direct Light).
4. HRP-Streptavidin (Ready to use, orange), 10 ml. 2-8°C (Avoid Direct Light).
5. TMB Substrate, 10 ml, 2-8°C (Avoid Direct Light)
6. Sample Dilution Buffer (blue), 20 ml, 2-8°C.
7. Stop Solution, 5 ml, 2-8°C.
8. Wash Buffer (25X), 30 ml, 2-8°C.
9. Plate Sealer, 5 pieces.

Materials Required But Not Supplied

1. Microplate reader (wavelength: 450 nm)
2. 37°C incubator (When using a water bath incubator, ensure the internal air temperature is 35-38°C; When using the cell CO2 incubator, use a sealed bag to isolate the microplate)
3. Automated plate washer or multi-channel pipette/5 ml pipettor (for manual washing purpose)
4. Precision single (0.5-10 µL, 5-50 µL, 20-200 µL, 200-1000 µL) and multi-channel pipette with disposable tips(calibration is required before use.)
5. Sterile tubes and Eppendorf tubes with disposable tips
6. Absorbent paper and loading slot
7. Deionized or distilled water

Storage

2-8°C (for sealed box), please do not freeze! See kit label for expiry date.

Specimen Collection And Preparation

Samples needed for single well(Max): Serum: 50 µl, Plasma: 50 µl, Cell Culture Supernatant: 50 µl, cell or tissue lysate: 50 µl, Other liquid samples: 50 µl.

1. **Serum**

Place whole blood sample at room temperature for 2 hours or at 2-8°C overnight. Centrifuge for 20min at 1000 xg and collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay.

2. **Plasma**

EDTA-Na2/K2 is recommended as the anticoagulant. Centrifuge samples for 15 minutes at 1000 ×g 2-8°C within 30 minutes after collection. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay. For other anticoagulant types and uses, please refer to the sample preparation guideline.

3. **Tissue Sample**

Generally tissue samples are required to be made into homogenization. Protocol is as below:

3.1. Place the target tissue on the ice. Remove residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Then weigh for usage.

3.2. Use lysate to grind tissue homogenates on the ice. The adding volume of lysate depends on the weight of the tissue. Usually, 9 mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS (e.g. 1 mM PMSF).

3.3. Do further process using ultrasonic disruption or freeze-thaw cycles (Ice bath for cooling is required during ultrasonic disruption; Freeze-thaw cycles can be repeated twice.) to get the homogenates.

3.4. Homogenates are then centrifuged for 5 minutes at 5000 ×g. Collect supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay.

3.5. Determine total protein concentration by BCA kit for further data analysis. Usually, total protein concentration for Elisa assay should be within 1-3 mg/ml. Some tissue samples such as liver, kidney, pancreas which containing a higher endogenous peroxidase concentration may react with TMB substrate causing false positivity. In that case, try to use 1% H2O2 for 15min inactivation and perform the assay again.

Notes: PBS buffer or the mild RIPA lysis can be used as lysates. While using RIPA lysis, make the PH=7.3. Avoid using any reagents containing NP-40 lysis buffer, Triton X-100 surfactant, or DTT due to their severe inhibition for kits' working. We recommend using 50 mM Tris+0.9%NaCL+0.1%SDS, PH 7.3. You can prepare by yourself or contact us for purchasing.

4. **Cell Culture Supernatant**

Collect the supernatant: Centrifuge at 2500 rpm at 2-8°C for 5 minutes, then collect clarified cell culture supernatant to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

5. **Cell Lysate**

5.1.Suspension Cell Lysate: Centrifuge at 2500 rpm at 2-8°C for 5 minutes and collect cells. Then add pre-cooling PBS into collected cell and mix gently. Recollect cell by repeating centrifugation. Add 0.5-1 ml cell lysate and appropriate protease inhibitor (e.g. PMSF, working concentration: 1mmol/L). Lyse the cell on ice for 30 min-1 h or disrupt the cell by ultrasonic disruption.

5.2. Adherent Cell Lysate: Absorb supernatant and add pre-cooling PBS to wash three times. Add 0.5-1 ml cell lysate and appropriate protease inhibitor (e.g. PMSF, working concentration: 1 mmol/L). Scrape the adherent cell with cell scraper. Lyse the cell suspension added in the centrifuge tube on ice for 30 min-1 h or disrupt the cell by ultrasonic disruption.

5.3. During lysate process, use the tip for pipetting or intermittently shake the centrifugal tube to completely lyse the protein. Mucilaginous product is DNA which can be disrupted by ultrasonic cell disruptor on ice. (3-5 mm probe, 150-300 W, 3-5s/time, 30s intervals for 1-2s working).

5.4. At the end of lysate or ultrasonic disruption, centrifuge at 10000 rpm at 2-8°C for 10 minutes. Then, the supernatant is added into EP tube to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

Notes: Read notes in tissue sample. Determine total protein concentration by BCA kit for further data analysis. Usually, total protein concentration for Elisa assay should be within 1-3 mg/ml.

6. **Other Biological Sample**

Centrifuge samples for 15 minutes at 1000 ×g at 2-8°C. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

Notes for Samples

1. Blood collection tubes should be disposable and non-endotoxin. Avoid to use hemolyzed and lipemia samples.
2. The best sample storage condition: less than 5 days at 2-8°C; within 6 months at -20°C; within 2 years at -80°C. Stored in liquid nitrogen for a longer storage. When melting frozen samples, rapid water bath at 15-25°C can decrease the effect of ice crystal (0°C) on the sample. After melting, centrifuge to remove the precipitate, and then mix well.
3. The detection range of this kit is not equivalent to the concentration of analyze in the sample. For analyses with higher or lower concentration, please properly dilute or concentrate the sample.
4. Pretest is recommended for special samples without reference data to validate the validity.

5. Recombinant protein may not match with the capture or detection antibody in the kit, resulting in the undetectable assay.

Recommended reagents for sample preparation: 100 mM PMSF protease inhibitor, Lysis Buffer (for ELISA).

Recommended Sample Dilution Ratio

Please refer to the following table of recommended dilution ratio for limited samples for reference. (ND: Not Detected) (When the concentration of the target in sample is very low, the sample can be added directly without dilution.)

Sample Type ----- Recommended Dilution Ratio ------ Content

Healthy serum ------ undiluted ------- 0.52-5.84 ng/ml

A-431 cell lysate ------ undiluted ------ 10.5 ng/mg (total protein)

If other dilution ratio for your sample model is required, please refer to the universal dilution ratio below. (The ratio is suitable for single-well assay. For duplicate assay, please follow the calculation: volume of sample and diluent x number of duplicate well)

For 2 fold dilution (1/2): One step dilution. Add 60 µl sample into 60 µl sample diluent and mix gently.

For 5 fold dilution (1/5): One step dilution. Add 24 µl sample into 96 µl sample diluent and mix gently.

For 10 fold dilution (1/10): One step dilution. Add 12 µl sample into 108 µl sample diluent and mix gently.

For 20 fold dilution (1/20): One step dilution. Add 6 µl sample into 114 µl sample diluent and mix gently.

For 50 fold dilution (1/50): One step dilution. Add 3 µl sample and 47 µl normal saline (0.9% NaCl) into 100 µl sample diluent and mix gently.

For 100 fold dilution (1/100): One step dilution. Add 3 µl sample and 177 µl normal saline into 120 µl sample diluent and mix gently.

For 1000 fold dilution (1/1000): Two step dilution. Create a 50-fold dilution first (normal saline is used throughout the dilution). Then, create a 20-fold dilution and mix gently.

For 10000 fold dilution (1/10000): Two step dilution. Create a 100-fold dilution first (normal saline is used throughout the dilution). Then, create the same dilution again and mix gently.

For 100000 fold dilution (1/100000): Three step dilution. Create a 50-fold dilution and 20-fold dilution respectively (normal saline is used in the first two steps.) Finally, create a 100-fold dilution and mix gently.

Notes: The volume in each dilution is not less than 3µl. Dilution factor should be within 100 fold. Mixing during dilution is required to avoid foaming.7

Reagent Preparation

Take the Elisa kit from the fridge around 20 minutes earlier and equilibrate to room temperature(18-25°C). For repeated assays, please just take the strips and standards required for the current assay, store the rest materials according to the relevant condition.

1. **Wash Buffer**

Dilute 30 ml concentrated wash buffer to 750 ml wash buffer with deionized or distilled water and mix well. (The recommended resistivity of ultrapure water is 18 MΩ.) Alternatively, take appropriate amount of concentrated wash buffer according to the assay requirement, then create a 25-fold dilution and mix well.

Store the rest solution at 2-8°C.

Crystals formed in the concentrated wash buffer can be heated by water bath at 40°C till complete dissolution. (Heating temperature should be below 50°C.) Mix well for the next step. It's better to use up the prepared wash buffer in one day. Store the rest buffer at 2-8°C within 48h.

2. **Sample dilution**

2.1. The "Recommended Sample Dilution Ratio" in the manual refers to the recommended dilution ratio for limited samples like normal serum, normal plasma, cell lysate or cell culture supernatant, not indicating the proteins' expression status in all the samples. Due to disease or model process, the optimal dilution ratio of your samples may be different from the recommended dilution ratio in the manual. To avoid experimental failure caused by unsuitable sample dilution ratio, it is recommended to carry out pre-experiment before formal assay, by selecting a small amount of samples from different groups and considering the "Recommended Sample Dilution ratio" and the corresponding disease or model treatment, setting 3 to 4 groups with 10-fold dilution to get the optimal dilution ratio. For example, target protein A in normal serum needs to be diluted at 1/100 and the disease will lead to its decrease, then you can set four gradients (1/10, 1/100, 1/1000, and 1/10000) while pre-experiment.

2.2. Please refer to "Recommended Sample Dilution Ratio" in the manual to learn operations in detail.

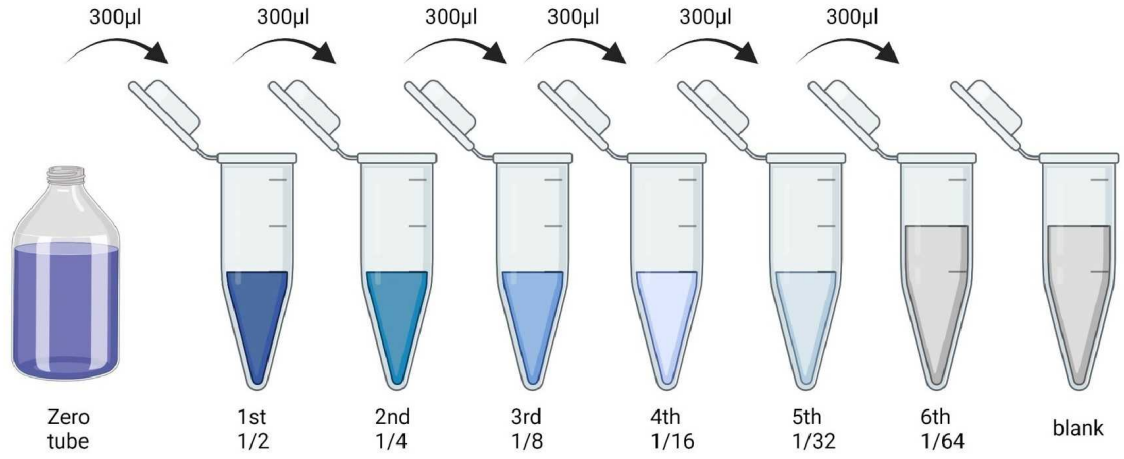
3. **Standards**

3.1. Centrifuge standards tube for 1min at 10000 xg. Label it as Zero tube.

3.2. Add 0.5 ml sample dilution buffer into the standard tube. Tighten the tube cap and Let it stand for 2 min at room temperature. Invert the tube several times to mix gently. (Or you can mix it using a low speed vortex mixer for 3-5 seconds.)

3.3. Centrifuge the tubes for 1min at 1000 xg, making the liquid towards the bottom of tube and removing possible bubbles.

3.4. Standard dilution: Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 150 µl of the sample dilution buffer into each tube. Add 150ul solution from zero tube into 1/2 tube and mix them thoroughly. Transfer 150 µl from 1/2 tube into 1/4 tube and mix them thoroughly. Transfer 150 µl from 1/4 tube into 1/8 tube and mix them thoroughly, so on till 1/64 tube. Now blank tube only contain 150 µl sample dilution buffer. The standard concentration from zero tube to blank tube is 2X 20 ng/ml, 20 ng/ml, 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0 ng/ml.



Notes: Store the zero tube with dissolved standards at 2-8°C and use it within 24 h(Do not freeze). Other diluted working solutions containing standards should be used in 2 h.

Assay Procedure

(It is necessary to continuously finish the whole experimental process. The reaction wells need to be immediately added with the working solution, which cannot be too dry. Otherwise, it might not work properly.)

When diluting samples, they must be mixed completely. It's recommended to plot a standard curve for each test.

1. Set standard, pilot samples, control (blank) wells on the pre-coated plate respectively, and then, records their positions. It's recommended to measure each standard and sample in duplicate to decrease experimental errors.
2. Cap/Det Ab and Standards/sample loading: Add 50 µl Cap/Det Ab into each well. Aliquot 50 µl of zero tube, 1/2 tube, 1/4 tube, 1/8 tube, 1/16 tube, 1/32 tube, 1/64 tube and blank into each standard well. Then, add 50 µl pilot samples into sample wells. Immediately, gently tap the plate for 10s to ensure thorough mixing then static incubate for 60 minutes at 37°C. (When adding standard or sample, the disposable tip lightly touches the liquid level. Change the disposable tips for different samples and standards.)
3. Wash twice: Remove the cover, then absorb the liquid in the plate or tap the plate on a clean absorbent paper two or three times. Add 300-350 µl wash buffer into each well without immersion. Discard the liquid in the well and tap on the absorbent paper again. Repeat the washing step twice.
4. HRP-Streptavidin: Add 100 µl HRP-Streptavidin into each well. Seal the plate and static incubate for 30 minutes at 37°C. (Put the whole bottle of TMB into room temperature for 30 min.)
5. Wash five times: Remove the cover, and then wash the plate with wash buffer five times. Read washing method in step 3.
6. TMB Substrate: Add 90 µl TMB Substrate into each well, seal the plate and static incubate at 37°C in dark within 10-20 minutes. Run the microplate reader and preheat for 15min. (Notes: Please do not use the reagent reservoirs used by HRP couplings. The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells. Weaker or stronger color intensity is unacceptable.)
9. Stop: Keep the liquid in the well after staining. Add 50ul stop solution into each well. The color will turn yellow immediately. The order for adding stop solution and TMB substrate solution is the same.
10. OD Measurement: Read the O.D. absorbance at 450 nm in a microplate reader immediately. (If your microplate reader has a choice of correction wavelength, set it to 570 nm or 630nm. Correct the read value to the OD450 value minus the OD570 or OD630 value. In this way, the OD value of non-chromogenic substances can be corrected and removed, thus obtaining more accurate results. If the microplate reader does not have a 570 nm or 630 nm wavelength, the original OD450 value can be used.)

Assay Procedure Summary

Step 1: Take out the required plate wells, add 50 µl Cap/Det Ab into each well, then add 50 µL Standard or Sample into individual well. (When adding standard or sample, the disposable tip lightly touches the liquid level. Change the disposable tips for different samples and standards.) Gently tap the plate for 10s to ensure thorough mixing then static incubate for 60 minutes at 37°C.

Washing: Wash the plate twice without immersion.

Step 2: Add 100 µl HRP-Streptavidin (orange) into each well, seal the plate and static incubate for 30 minutes at 37°C.

Washing: Wash the plate five times without immersion.

Step 3: Add 90 µl TMB substrate solution, seal the plate and static incubate for 10-20 minutes at 37°C.

Step 4: Add 50 µl stop solution. Read at 450 nm immediately and calculate.

Calculation

1. Calculate the mean OD450 value (using the original OD450 value or the corrected OD450 value) of the duplicate readings for each standard, control, and sample. Then, obtain the value of calculation by subtracting the OD450 blank.
2. Create a four parameter logistic curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis. (Remove the OD450 blank during plotting.) Alternatively, you can use the curve fitting software offered by the microplate reader (e.g. Thermo SkanIt RE software, Curve Expert 1.3 or 1.4).
3. Calculate the sample concentration by substituting OD450 value into the standard curve.

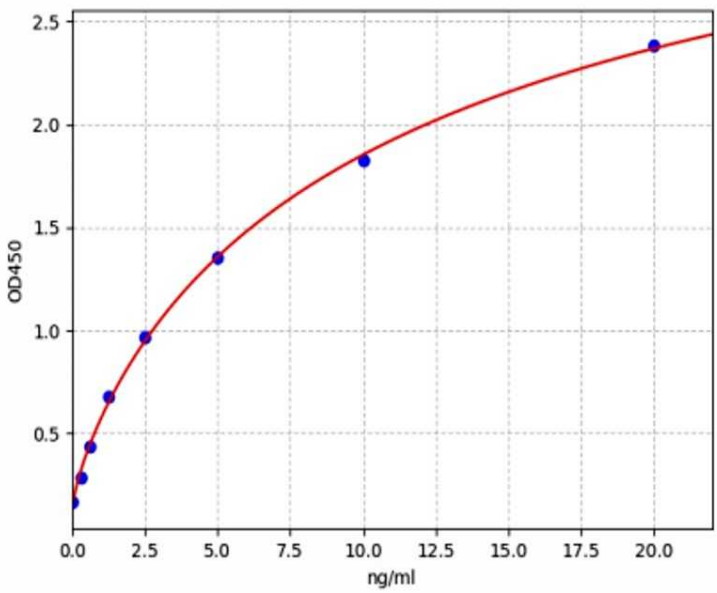
Note: If the sample is added undiluted, the sample dilution caused by incubation of both the sample and antibody should be considered. In this case, the final concentration should be multiplied by 2 from the calculated value. If the sample is 1/100 diluted before adding the plate wells, the final concentration should be multiplied by 200 from the calculated value. And so on.

Typical Standard Curve

This product has been tested by Quality Control Department and meets performance specifications mentioned in the manual. (The humidity in the laboratory is 20%-60%, and the temperature is 18°C -25°C. (TMB was balanced to room temperature before color development, and incubated at 37°C for 15 minutes in the dark after adding the enzyme label plate holes.)

The following assay data are provided for reference, since experimental environment and operation are different. The establishment of standard curve depends on your own assay.

STD.(ng/ml)	OD-1	OD-2	Average	Corrected
0	0.163	0.167	0.165	0
0.312	0.286	0.282	0.285	0.12
0.625	0.438	0.428	0.433	0.268
1.25	0.745	0.739	0.713	0.548
2.5	1.078	1.07	1.023	0.858
5	1.282	1.291	1.318	1.153
10	1.893	1.911	1.861	1.696
20	2.464	2.405	2.423	2.258



Precision

Intra-assay Precision: samples with low, medium and high concentration are tested 20 times on same plate.

Inter-assay Precision: samples with low, medium and high concentration are tested 20 times on three different plates.

Item	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/ml)	0.57	2.6	10.75	0.58	2.5	9.35
Standard deviation	0.02	0.09	0.43	0.02	0.11	0.4
CV(%)	3.98	3.56	4.02	4.31	4.42	4.25

Detection Range

0.313-20 ng/ml

Sensitivity

0.188 ng/ml

Specificity

Specifically recognize TDP43, no obvious cross reaction with other analogues.

Linearity

Dilute the sample with a certain amount of TDP43 at 1:2, 1:4 and 1:8 to get the recovery range.

Sample	1:2	1:4	1:8
Serum(n=5)	85-104%	83-96%	80-97%
EDTA Plasma(n=5)	87-105%	85-98%	83-100%
Heparin Plasma(n=5)	83-98%	80-95%	87-100%

Recovery

Add a certain amount of TDP43 into the sample. Calculate the recovery by comparing the measured value with the expected amount of TDP43 in the sample.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	86-101	99
EDTA Plasma(n=5)	91-100	95
Heparin Plasma(n=5)	89-101	99

Precautions

1. When using different Elisa kits, labeling is required to avoid mixed components and failed assay.
2. After opening the kit, please refer to the table of storage condition for coated plate and standards (Dampness may decrease the activity.). If any component is missing or damaged during the assay or storage, please contact us for ordering a new one to replace.
3. Sterile and disposable tips are required during the assay. After use, the reagents bottle cap has to be tightened to avoid the microbial contamination and evaporation.
4. While manual washing, please keep tips or pipettors for adding wash buffer away from the well. Insufficient washing or contamination easily causes false positive and high background.
5. During the assay, prepare required reagents for next step in advance. After washing, add the reagent into the well in time to avoid dryness. Otherwise, dry plate will result in the failed assay.
6. Before confirmation, reagents from other batches or sources should not be used in this kit.
7. Don't reuse tips and tubes to avoid cross contamination.
8. After loading, seal the plate to avoid the evaporation of the sample during incubation. Complete the incubation process at recommended temperature.
9. Please wear the lab coat, mask and gloves to protect yourself during the assay. Especially, for the detection of blood or other body fluid sample, please follow regulations on safety protection of biological laboratory.