



**Cat. No.: RSHAKRIE010R**

This kit is manufactured by Shibayagi Co., Ltd.

Use only the current version of Instruction Manual enclosed with the kit!

For the detailed assay procedure, refer to [Key points for ELISA by movie](#) on our website: <http://www.shibayagi.co.jp/index-E.htm>

## 1. INTENDED USE

Mouse IgE ELISA Kit is a sandwich ELISA system for quantitative measurement of mouse IgE. This is intended for research use only.

## 2. STORAGE AND EXPIRATION

When the complete kit is stored at 2-8°C, the kit is stable until the expiration date shown on the label on the box. Opened reagents should be used as soon as possible to avoid loss in optimal assay performance caused by storage environment.

## 3. INTRODUCTION

IgE (Immunoglobulin E) is a glycoprotein with a molecular size of 190 kDa composed of 2 light chains and 2 heavy chains (H $\epsilon$ ). In electrophoresis, it moves to  $\gamma$ 1 region. Its biological half life is about 3 days, and its blood level in normal human subject is very low, about 300 ng/ml. The blood level of IgE increases markedly in parasite infection and in hay fever. IgE that is responsible for allergy has been called “reagin”. Sensitization by an allergen increases reagin IgE which binds to Fc $\epsilon$ R1 receptor in basophilic leucocytes and mast cells at Fc region and sensitizes those cells. If the allergen binds the sensitized cells, those cells will be

degranulated and release histamin, serotonin, protease, heparin, chemotactic factor, prostaglandins, leucotriens, and so on, causing bronchoconstriction, mucous edema, and hypersecretion, and leads to type I allergic reactions like bronchial asthma, hives, allergic rhinitis, anaphylaxis, and so on. This is the assay kit for total mouse IgE. Shibayagi is also providing assay kits for allergen(OVA)-specific mouse IgE.

#### 4. ASSAY PRINCIPLE

In Shibayagi's Mouse IgE ELISA Kit, standards or samples are incubated in monoclonal antibody-coated wells to capture IgE. After 2 hours' incubation and washing, biotin-conjugated anti-IgE antibody is added and incubated further for 2 hours to bind with captured IgE. After washing, HRP- (horse radish peroxidase) conjugated avidin is added, and incubated for 1 hour. After washing, bound HRP-conjugated avidin is reacted with a chromogenic substrate reagent (TMB) for 20 minutes, and reaction is stopped by addition of acidic solution, and absorbance of yellow product is measured spectrophotometrically at 450 nm. The absorbance is nearly proportional to IgE concentration. The standard curve is prepared by plotting absorbance against standard IgE concentrations. IgE concentrations in unknown samples are determined using this standard curve.

#### 5. PRECAUTIONS

- For professional use only. Beginners are advised to use this kit under the guidance of experienced person.
- Do not drink, eat or smoke in the areas where assays are carried out.
- In treating assay samples of animal origin, be careful for possible biohazards.
- This kit contains components of animal origin. These materials should be handled as potentially infectious.
- Be careful not to allow the reagent solutions of the kit to touch the skin, eyes and mucus membranes. Especially be careful for the reaction stopper because it is 1 M sulfuric acid. The reaction stopper and the substrate solution may cause skin/eyes irritation. In case of contact with these wash skin/eyes thoroughly with water and seek medical attention, when necessary.
- Avoid contact with the acidic Reaction stopper solution and Chromogenic substrate reagent containing hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents.
- The materials must not be pipetted by mouth.

- Unused samples and used tips should be rinsed in 1% formalin, 2% glutal aldehyde, or more than 0.1% sodium hypochlorite solution for more than 1 hour, or be treated by an autoclave before disposal.
- Dispose consumable materials and unused contents in accordance with applicable regional/national regulatory requirements.
- Use clean laboratory glassware.
- In order to avoid dryness of wells, contamination of foreign substances and evaporation of dispensed reagents, never forget to cover the well plate with a plate cover supplied, during incubation.
- ELISA can be easily affected by your laboratory environment. Room temperature should be at 20-25°C strictly. Avoid airstream velocity over 0.4 m/sec. (including wind from air conditioner), and humidity less than 30%. For more details, watch our web movie [\[Assay circumstance\]](#).

## 6. REAGENTS SUPPLIED

| Components   | State                                | Amount           |
|--|--------------------------------------|------------------|
| A. Antibody-coated plate   | Use after washing                    | 96 wells/1 plate |
| B. IgE Standard solution (100 ng/ml)<br>(derived from mouse)                   | Concentrated.<br>Use after dilution  | 600 µl/1 vial    |
| C. Buffer solution   | Ready for use.                       | 60 ml/1 bottle   |
| D. Biotin-conjugated anti-IgE antibody   | Concentrated.<br>Use after dilution. | 10 µl/1 vial     |
| E. HRP-conjugated avidin   | Concentrated.<br>Use after dilution. | 20 µl/1 vial     |
| F. Chromogenic substrate reagent (TMB)   | Ready for use.                       | 10 ml/1 bottle   |
| G. Reaction stopper (1M H <sub>2</sub> SO <sub>4</sub> )<br><b>Be careful!</b> | Ready for use.                       | 10 ml/1 bottle   |
| H. Concentrated washing buffer (10x)   | Concentrated.<br>Use after dilution. | 100 ml/1 bottle  |
| Plate seal   | —                                    | 4 sheets         |
| Instruction Manual   | —                                    | 1 copy           |

## 7. EQUIPMENTS OR SUPPLIES REQUIRED BUT NOT SUPPLIED

Use as a check box

- Purified water (distilled water)
- Test tubes for preparation of standard solution series.
- Glassware for dilution of washing buffer (a graduated cylinder, a bottle)
- Pipettes (disposable tip type). One should be able to deliver 5 µl precisely, and another for 10-100 µl and 100-500 µl.
- Syringe-type repeating dispenser like Eppendorf multipipette plus which can dispense 50 µl.
- Paper towel to remove washing buffer remaining in wells.
- A vortex-type mixer.
- A shaker for 96 well-plate (600-1200 rpm)
- An automatic washer for 96 well-plate (if available), or a wash bottle with a jet nozzle (refer to our web movie [\[Washing of microplate\]](#)).
- A 96 well-plate reader (450 nm ±10 nm, 620 nm: 600-650 nm)
- Software for data analysis, if available. Shibayagi is proposing the use of assay results calculation template for EXCEL. Please check our website ([http://www.shibayagi.co.jp/en/tech\\_003.html](http://www.shibayagi.co.jp/en/tech_003.html)).

## 8. PREPARATION OF REAGENTS

Bring all reagents of the kit to room temperature (20-25°C) before use.

Prepare reagent solutions in appropriate volume for your assay. Do not store the diluted reagents.

### Concentrated reagents

#### (B) IgE Standard solution (100 ng/ml)

Make a serial dilution of master standard (100 ng/ml) solution to prepare each standard solution.

| Volume of standard solution | Buffer solution | Concentration (ng/ml) |
|-----------------------------|-----------------|-----------------------|
| Original solution           | 0 µl            | 100                   |
| Original solution 150 µl    | 50 µl           | 75                    |
| Original solution 100 µl    | 100 µl          | 50                    |
| Original solution 50 µl     | 150 µl          | 25                    |
| Original solution 20 µl     | 180 µl          | 10                    |
| Original solution 5 µl      | 495 µl          | 1.0                   |
| 0 (Blank)                   | 200 µl          | 0                     |

(D) Biotin-conjugated anti-IgE antibody

Prepare working solution by dilution of (D) with the buffer solution (C) to **1:1000**.

(E) HRP-conjugated avidin

Prepare working solution by dilution of (E) with the buffer solution (C) to **1:2000**.

(I) Concentrated washing buffer (10x)

Dilute 1 volume of the concentrated washing buffer (10x) to **10 volume** with deionized water to prepare working solution. Example: 100 ml of concentrated washing buffer (10x) and 900 ml of deionized water.

**Storage and stability**

(A) Antibody-coated plate

If seal is not removed, put the strip back in a plastic bag with zip-seal originally used for well-plate container and store at 2-8°C. The strip will be stable until expiration date.

(B) IgE Standard solution (100 ng/ml)

Standard solutions prepared above should be used as soon as possible, and should not be stored.

The rest of original standard: if stored tightly closed at 2-8°C, it is stable until expiration date.

(C) Buffer solution & (F) Chromogenic substrate reagent

If not opened, store at 2-8°C. It maintains stability until expiration date. Once opened, we recommend using as soon as possible to avoid influence by environmental condition.

(D) Biotin-conjugated anti-IgE antibody & (E) HRP-conjugated avidin

Unused working solution (already diluted) should be disposed. The rest of the undiluted solution: if stored tightly closed at 2-8°C, it is stable until expiration date.

(H) Reaction stopper (1 M H<sub>2</sub>SO<sub>4</sub>)

Close the stopper tightly and store at 2-8°C. It maintains stability until expiration date.

### (I) Concentrated washing buffer (10x)

The rest of undiluted buffer: if stored tightly closed at 2-8°C, it is stable until expiration date.

Dispose any unused diluted buffer.

## **9. TECHNICAL TIPS**

- In manual operation, proficiency in pipetting technique is recommended.
- The reagents are prepared to give accurate results only when used in combination within the same box. Therefore, do not combine the reagents from kits with different lot numbers. Even if the lot number is the same, it is best not to mix the reagents with those that have been preserved for some period.
- Be careful to avoid any contamination of assay samples and reagents. We recommend the use of disposal pipette tips, and 1 tip for 1 well.
- Optimally, the reagent solutions of the kit should be used immediately after reconstitution. Otherwise, store them in a dark place at 2-8°C.
- Time the reaction from the pipetting of the reagent to the first well.
- Prepare a standard curve for each assay.
- Dilution of the assay sample must be carried out using the buffer solution provided in the kit.
- The chromogenic substrate reagent (TMB) should be almost colorless before use. It turns blue during reaction, and gives yellowish color after addition of reaction stopper. Greenish color means incomplete mixing.
- To avoid denaturation of the coated antibody, do not let the plate go dry.
- As the antibody-coated plate is module type of 8wells x 12 strips, each strip can be separated by cutting the cover sheet with a knife and used independently.
- When ELISA has to be done under the airstream velocity of over 0.4 m/sec. and the humidity of less than 30%, completely close each well in addition to cover the well plate with a plate cover in each step of incubation.  
Ex.) Cover the well plate with parafilm, and put the plate cover on it. Or place the well plate with the plate cover in an incubator, or in a styrofoam box. Take the best way depending on situation of each laboratory. For more details, watch our web movie [\[Assay circumstance\]](#).

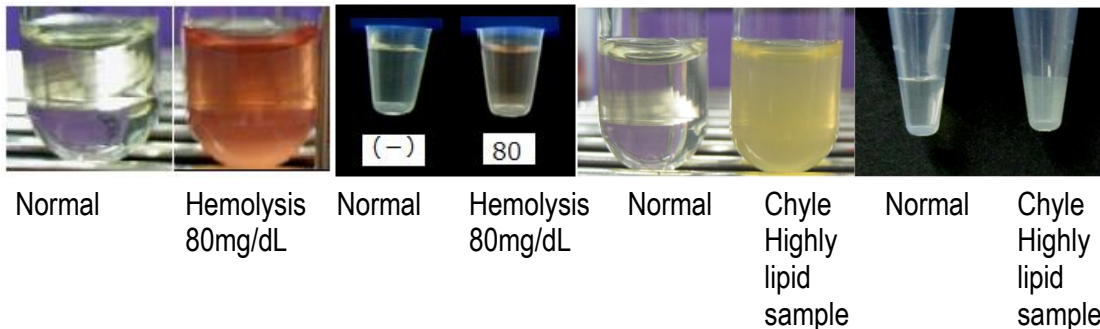
## 10. PREPARATION OF SAMPLES

This kit is intended to measure IgE in mouse serum or plasma. The necessary sample volume for the standard procedure is 5  $\mu$ l.

Samples should be immediately assayed or stored below  $-35^{\circ}\text{C}$  until assay. Dilution of a sample should be made in a test tube using buffer solution prior to adding them to wells. Turbid samples or those containing insoluble materials should be centrifuged before testing to remove any particulate matter.

[Hemolytic and hyperlipemic serum samples are not suitable.](#)

\* To avoid influence of blood (high lipid or hemolysis, etc.), if your original samples have heavy chyle or hemolysis as the pictures below, do not use them for assay. Abnormal value might be obtained with hemolysis above 80mg/dL with this kit.



If presence of interfering substance is suspected, examine by dilution test at more than 2 points.

### Storage and stability

Use samples soon after collected. Collected samples can be stable within 1 week after collection if stored at  $2-8^{\circ}\text{C}$ . If you have to store assay samples for a longer period, snap-freeze samples and keep them below  $-35^{\circ}\text{C}$ . Defrosted samples should be mixed thoroughly for best results. Avoid repeated freeze-thaw cycles.

## 11. ASSAY PROCEDURE

Remove the cover sheet of the antibody-coated plate after bringing up to room temperature.

1. Wash the antibody-coated plate (A) by filling the wells with washing buffer and discard 3 times(\*②), then strike the plate upside-down onto several sheets of paper towel to remove residual buffer in the wells.
2. Pipette 50 µl of properly diluted samples to the designated sample wells.
3. Pipette 50 µl of standard solution to the wells designated for standards.
4. Shake the plate gently on a plate shaker(\*③).
5. Stick a plate seal (\*④) on the plate and incubate for 2 hours at 20-25°C.
6. Discard the reaction mixture and rinse wells as step (1).
7. Pipette 50 µl of Biotin-conjugated anti-IgE antibody to all wells, and shake as step (4).
8. Stick a plate seal (\*④) on the plate and incubate the plate for 2 hours at 20-25°C.
9. Discard the reaction mixture and rinse wells as step (1).
10. Pipette 50 µl of HRP-conjugated avidin to all wells, and shake as step (4).
11. Stick a plate seal (\*④) on the plate and incubate the plate for 1 hour at 20-25°C.
12. Discard the reaction mixture and rinse wells as step (1).
13. Pipette 50 µl of Chromogenic substrate reagent to wells, and shake as step (4).
14. Stick a plate seal (\*④) on the plate and incubate the plate for 20 minutes at 20-25°C.
15. Add 50 µl of the reaction stopper to all wells and shake as step (4).
16. Measure the absorbance of each well at 450 nm (reference wavelength, 620nm) using a plate reader within 30 minutes.

\*Refer to the page 7-8 for notes of \*②, \*③ and \*④.



## 12. CALCULATIONS

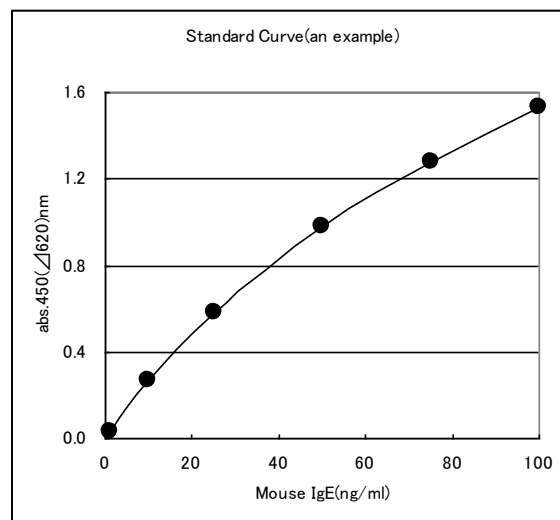
1. Prepare a standard curve by plotting standard concentration on X-axis and absorbance on Y-axis.  
(Refer to our web site for more detailed explanation about standard curve. Shibayagi is offering a convenient Excel template.

[http://www.shibayagi.co.jp/en/tech\\_003.html](http://www.shibayagi.co.jp/en/tech_003.html))

2. Using the standard curve, read the IgE concentration of a sample at its absorbance\*, and multiply the assay value by dilution factor if the sample has been diluted. Though the assay range is wide enough, in case the absorbance of some samples is higher than that of the highest standard, please repeat the assay after proper dilution of samples with the buffer solution.

\* We recommend the use of 3rd order regression curve for log-log plot, or 4 parameters method for log-normal plot in computer calculation.

Physiological or pathological situation of animals should be judged comprehensively taking other examination results into consideration.



Absorbance may change due to assay environment.

### 13. PERFORMANCE CHARACTERISTICS

- Assay range

The assay range of the kit is 1 ng/ml ~ 100 ng/ml.

- Specificity

The antibodies used in this kit are specific to IgE. Cross-reactivity of the kit is shown below.

| Substances | Cross-reactivity | Concentration |
|------------|------------------|---------------|
| Mouse IgE  | 100%             | 100 ng/ml     |
| Mouse IgG  | < 0.01%          | 1 mg/ml       |
| Mouse IgA  | < 0.01%          | 1 mg/ml       |
| Mouse IgM  | < 0.01%          | 1 mg/ml       |
| Rat IgE    | < 0.1%           | 100 ng/ml     |
| Human IgE  | < 0.1%           | 100 ng/ml     |
| BSA        | < 0.01%          | 10 mg/ml      |

- Precision of assay

Within assay variation (3 samples, 5 replicates assay,) Mean CV is less than 5%.

- Reproducibility

Between assay variation (3 samples, 4 days, 5 replicates assay) Mean CV is less than 5%

- Recovery test

Standard IgE was added in 2 concentrations to 2 serum samples and were assayed.

The recoveries were 99.7 ~103%

- Dilution test

Serum sample was serially diluted by 3 steps.

The dilution curves showed linearity with  $R^2 = 0.999$ .

## 14. REFERENCE ASSAY DATA

Mouse IgE assay data

**Mean assay value: 85 ng/ml, SD: 18 ng/ml**

Mouse strains: NC/Nga, female, 5 weeks-old

Number of animals: 24 Samples: serum

These data should be considered as guidance only. Each laboratory should establish its own normal and pathological reference ranges for IgE levels independently.

## 15. TROUBLE SHOOTING

- Low absorbance in all wells

Possible explanations:

- 1) The standard or samples might not be added.
- 2) Reagents necessary for coloration such as Biotin-conjugated anti-IgE antibody, HRP-conjugated avidin, or Chromogenic substrate reagent might not be added.
- 3) Wrong reagents related to coloration might have been added. Wrong dilution of biotin-conjugated anti- IgE antibody or HRP-conjugated avidin.
- 4) Contamination of enzyme inhibitor(s).
- 5) Influence of the temperature under which the kits had been stored.
- 6) Excessive hard washing of the well plate.
- 7) Addition of chromogenic substrate reagent soon after taking out from a refrigerator might cause poor coloration owing to low temperature.

- Blank OD was higher than that of the lowest standard concentration (1.0 ng/ml).

Possible explanations:

Improper or inadequate washing. (Change washing frequency from 3 times to 4-6 times at the constant stroke after the reaction with HRP-conjugated avidin.)

- High coefficient of variation (CV)

Possible explanation:

- 1) Improper or inadequate washing.
- 2) Improper mixing of standard or samples.
- 3) Pipetting at irregular intervals.

- Q-1: Can I divide the plate to use it for the other testing?

A-1: Yes, cut off the clear seal on the plate with cutter along strip. Put the residual plate, which is still the seal on, in a refrigerator soon.

● Q-2: I found there contains liquid in 96 well-plate when I opened the box. What is it?

A-2: When we manufacture 96 well-plate, we insert preservation stabilizer in wells.

For detailed FAQs and explanations, refer to “**Trouble shooting and Important Points in Shibayagi’s ELISA kits**” on our website

([http://www.shibayagi.co.jp/en/tech\\_004.html](http://www.shibayagi.co.jp/en/tech_004.html)).

## 16. REFERENCES

Please, refer to [\[User’s Publication\]](#) on our website.

**Summary of assay procedure:** Use as a check box

**\*First, read this instruction manual carefully and start your assay after confirmation of details.**

For more details, watch our web movie [\[ELISA by MOVIE\]](#) on our website.

Bring the well-plate and all reagents back to **20-25°C for 2 hours**.

Concentrated washing buffer must be diluted to **10 times** by purified water that returned to 20-25°C.

IgE Standard solution dilution example:

|                                  |     |     |     |     |     |     |     |
|----------------------------------|-----|-----|-----|-----|-----|-----|-----|
| Concentration (ng/ml)            | 100 | 75  | 50  | 25  | 10  | 1.0 | 0   |
| IgE Std. solution (μl)→ Ori.Sol. | 150 | 100 | 50  | 20  | 5   | 0   |     |
| Buffer solution (μl)             |     | 50  | 100 | 150 | 180 | 495 | 200 |

Prepare the positive sample.

## Precautions & related info

|                          |  |       |   |
|--------------------------|--|-------|---|
| <input type="checkbox"/> | Antibody-coated plate  |       |   |
| <input type="checkbox"/> | ↓Washing 3 times(*②)   |       | *⑥  |
| <input type="checkbox"/> | Diluted samples, or Standards  | 50 µl | *⑦ [Handling of pipetting]  |
| <input type="checkbox"/> | ↓Shaking(*③), Incubation for 2 hours at 20-25°C (Standing(*④))                               |       | *⑧ [Assay circumstance]   |
| <input type="checkbox"/> | Dilute Biotin-conjugated anti-IgE antibody (D) to 1000x with buffer (C) returned to 20-25°C. |       |   |
| <input type="checkbox"/> | ↓Washing 3 times(*②)   |       | *⑥  |
| <input type="checkbox"/> | Biotin-conjugated anti-IgE antibody solution   | 50 µl | *⑦ [Handling of pipetting]  |
| <input type="checkbox"/> | ↓Shaking(*③), Incubation for 2 hours at 20-25°C. (Standing(*④))                              |       | *⑧ [Assay circumstance]   |
| <input type="checkbox"/> | Dilute HRP-conjugated avidin (E) to 2000x with buffer (C) returned to 20-25°C.               |       |   |
| <input type="checkbox"/> | ↓Washing 3 times(*②)   |       | *⑥  |
| <input type="checkbox"/> | HRP conjugated avidin  | 50 µl | *⑦ [Handling of pipetting]  |
| <input type="checkbox"/> | ↓Shaking(*③), Incubation for 1 hour at 20-25°C. (Standing(*④))                               |       | *⑧ [Assay circumstance]   |
| <input type="checkbox"/> | ↓Washing 3 times(*②)   |       | *⑥  |
| <input type="checkbox"/> | Chromogenic substrate reagent (TMB)  | 50 µl | After dispense, the color turns to blue depending on the concentration.   |
| <input type="checkbox"/> | ↓Shaking(*③), Incubation for 20 minutes at 20-25°C. (Standing(*④))                           |       | *⑧ [Assay circumstance]   |
| <input type="checkbox"/> | Reaction stopper (1M H <sub>2</sub> SO <sub>4</sub> )  | 50 µl | After dispense, the color turns to yellow depending on the concentration. |
| <input type="checkbox"/> | ↓Shaking(*③)   |       | Immediately shake.  |
| <input type="checkbox"/> | Measurement of absorbance (450 nm, Ref 620 nm(*⑤))   |       | Ref. wave cancels the dirt in the back of plate.                          |

\*② After dispensing wash buffer to wells, lightly shake the plate on your palm for 10 sec and remove the buffer. Guideline of washing volume: 300µl/well for an automatic washer and for a pipette if the washing buffer is added by pipette. In case of washing by using 8 channel pipette, sometimes the back ground tends to be high. If so, change washing frequency from 3 times to 4-6 times at the constant stroke after the reaction with HRP conjugated streptavidin.

Standard of plate-washing pressure: 5-25ml/min. (Adjust it depending on the nozzle's diameter.) Refer to our web movie [\[Washing of microplate\]](#).

\*③ Guideline of shaking: **600-1,200 rpm for 10 seconds x 3 times.**

\*④ Put a plate cover on the plate during the reaction after shaking.

\*⑤ 600-650 nm can be used as reference wavelength.

\*⑥ After removal of wash buffer, immediately dispense the next reagent.

\*⑦ Refer to our web movie [\[Handling of pipetting\]](#).

\*⑧ Refer to our web movie [\[Assay circumstance\]](#).

### Worksheet example

|          | <b>Strip 1&amp;2</b> | <b>Strip 3&amp;4</b> | <b>Strip 5&amp;6</b> | <b>Strip 7&amp;8</b> | <b>Strip 9&amp;10</b> | <b>Strip 11&amp;12</b> |
|----------|----------------------|----------------------|----------------------|----------------------|-----------------------|------------------------|
| <b>A</b> | <b>100 ng/ml</b>     | Sample 1             | Sample 9             | Sample 17            | Sample 25             | Sample 33              |
| <b>B</b> | <b>75 ng/ml</b>      | Sample 2             | Sample 10            | Sample 18            | Sample 26             | Sample 34              |
| <b>C</b> | <b>50 ng/ml</b>      | Sample 3             | Sample 11            | Sample 19            | Sample 27             | Sample 35              |
| <b>D</b> | <b>25 ng/ml</b>      | Sample 4             | Sample 12            | Sample 20            | Sample 28             | Sample 36              |
| <b>E</b> | <b>10 ng/ml</b>      | Sample 5             | Sample 13            | Sample 21            | Sample 29             | Sample 37              |
| <b>F</b> | <b>1.0 ng/ml</b>     | Sample 6             | Sample 14            | Sample 22            | Sample 30             | Sample 38              |
| <b>G</b> | <b>0</b>             | Sample 7             | Sample 15            | Sample 23            | Sample 31             | Sample 39              |
| <b>H</b> | Positive Control     | Sample 8             | Sample 16            | Sample 24            | Sample 32             | Sample 40              |

### Assay worksheet

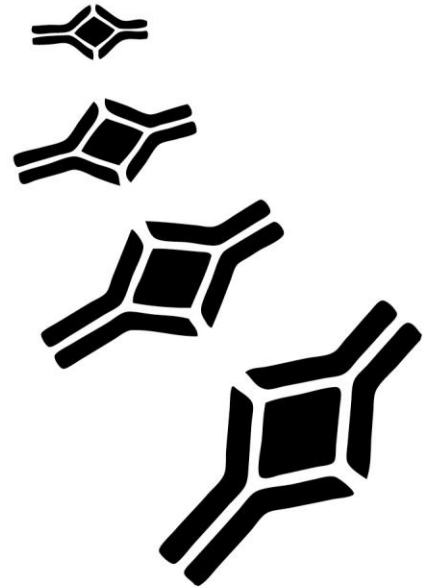
|   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|
| A |   |   |   |   |   |   |   |   |   |    |    |    |
| B |   |   |   |   |   |   |   |   |   |    |    |    |
| C |   |   |   |   |   |   |   |   |   |    |    |    |
| D |   |   |   |   |   |   |   |   |   |    |    |    |
| E |   |   |   |   |   |   |   |   |   |    |    |    |
| F |   |   |   |   |   |   |   |   |   |    |    |    |
| G |   |   |   |   |   |   |   |   |   |    |    |    |
| H |   |   |   |   |   |   |   |   |   |    |    |    |

#### Storage condition

Store the kit at 2-8°C (Do not freeze).

#### Term of validity

6 months from production (Expiration date is indicated on the container.)



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