

**Cat. No.: RSHAKMAN011R**

**For *in vitro* Laboratory Use Only**

**Please, read this instruction carefully before use.**

This is an ELISA (Enzyme Linked ImmunoSorbent Assay) kit for measurement of Mouse/Rat High Molecular Weight (HMW) Adiponectin with high sensitivity using sandwich assay principle.

## 1. ADVANTAGE

1. Rapid assay (total reaction time : 4 hours).
2. No sample pretreatment required.
3. A small sample volume.
4. An ecologically excellent preservative is used.
5. Every reagent is provided in liquid form and ready to use.
6. Excellent precision and reproducibility.

## 2. COMPONENTS

	Reagents	Amounts
A	Anti-adiponectin-coated plate	96 wells(8x12) / 1 plate
B	Standard adiponectin solution (2000ng/ml)	200 µl / 1 vial
C	Buffer solution	60ml/ 1 bottle
D	HRP-conjugated anti-adiponectin	100 µl / 1 vial
F	Chromogenic substrate reagent (TMB)	12ml/ 1 vial
H	Reaction stopper (1M H <sub>2</sub> SO <sub>4</sub> )	12ml/ 1 vial
I	Concentrated washing buffer (10x)	100ml/ 1 bottle

### 3. ASSAY SAMPLE

In most cases, samples should be diluted to 50X( ~25X ) using Buffer solution(C) as shown in the table below.

	<b>50-fold dilution</b>	<i>(25-fold dilution)</i>
Serum/Plasma (μl)	<b>10</b>	<i>(10)</i>
Buffer solution (μl)	<b>490</b>	<i>(240)</i>

### 4. ASSAY RANGE

3.13 ~ 200ng/ml (Standard curve)

(In the case of 50X dilution, original HMW adiponectin range is 156~10,000 ng/ml)

### 5. ASSAY OPERATION

#### 1. Equipments necessary but not included in the kit.

1. Micropipette (a micropipette able to deliver sample volume with high precision.), and a pipette for repetitive dispensing.
2. Microplate washing apparatus (a microplate washer or a flashing bottle with nozzle).
3. A microplate reader .

#### 2. Preparation of reagents

1. Washing buffer : Dilute the Concentrated washing buffer (I) to 10X with purified water.
2. HRP-conjugated anti-adiponectin (D) : Dilute to 100X with the Buffer solution(C).
3. Other reagents are used as they are.
4. All the reagent solutions should be used after bringing them up to room temperature (20-25°C).

### 3. An example of preparing standard solutions

Dilute the original Standard adiponectin solution (B) with the Buffer solution(C) to prepare 200ng/ml, then prepare lower standard solutions by a dilution program shown below. (You can use other mode of dilution for a set of standard solutions.)

Conc. (ng/ml)	200	100	50	25	12.5	6.25	3.13	0
Std. sol. (µl)	50**	200*	200*	200*	200*	200*	200*	0
Buffer sol. (µl)	450	200	200	200	200	200	200	200

\*\*Original standard solution, \*One rank higher standard solution

### 4. Assay procedure

\*Remove the cover sheet of the microplate after bringing reagents to room temperature.

1. Rinse the anti-adiponectin coated wells (A) 3 times by filling 350µl of the washing buffer and discarding the buffer. Remove residual buffer in the wells by striking the plate upside-down onto sheets of folded paper towel.
2. Pipette 50µl of diluted sample solution to those wells for samples,
3. Pipette 50µl of the standard solution to the assigned wells for preparing standard curve.
4. Shake the plate gently on a plate shaker at 600-1,000 rpm for 5-10 seconds.
5. Incubate for 2 hours at room temperature (20-25°C).
6. Discard the reaction mixture, and then wash the wells 3 times by filling 350µl of the washing buffer and discarding the buffer. Remove residual buffer in the wells by striking the plate upside-down onto sheets of folded paper towel.
7. Pipette 50µl of HRP-conjugated anti-adiponectin solution (D) to all wells. Then shake gently on a plate shaker.
8. Incubate the plate for 90 minutes at room temperature.
9. Discard the reaction mixture, and then wash the plate as in (6).
10. Pipette 50µl of Chromogenic substrate reagent(F) into wells, and shake as in (4).
11. Let the plate stand for 30 minutes at room temperature. Avoid exposing the microplate to sunlight.
12. Add 50 µl of the Reaction stopper (H) to all wells and shake.
13. Measure the absorbance of each well at 450 nm (sub-wave length, 620nm) by a micro-plate reader within 30 minutes.

## 6. SUMMARY OF ASSAY PROCEDURE

**Anti-adiponectin-coated plate**



Washing 3 times



**Sample or Standard      50μl**



Shaking, then reaction for 2 hours at room temp.



Washing 3 times



**HRP-conjugated anti-adiponectin      50μl**



Shaking, then reaction for 90 mins. at room temp.



Washing 3 times



**Chromogenic substrate solution      50μl**



Shaking, then reaction for 30 mins. at room temp



**Reaction stopper    1M H<sub>2</sub>SO<sub>4</sub>      50μl**



Shaking, then measurement of absorbance at 450nm(sub. 620nm)

Room temp. : 20~25°C

## 7. CALCULARION OF ADIPONECTIN (HMW) CONCENTRATION

1. Prepare a standard curve by plotting absorbance\* (Y-axis) against logarithmic standard concentration (ng/ml) on X-axis. For the manual reading from the standard curve, we recommend the use of bi-logarithmic section paper.  
\*Absorbance at 450nm minus absorbance at 620nm.
2. Adopt those diluted samples the absorbance of which are within the standard curve.
3. Read adiponectin (HMW) concentration of samples from their absorbance\*, and multiply the assay values by dilution rate. Though the assay range is wide enough, in case the absorbance of some samples are higher than that of the highest standard, please repeat the assay after proper dilution of samples with the buffer solution.
4. The assay values obtained with this kit cannot be directly compared with those obtained with mouse/rat adiponectin kits for total adiponectin because of the difference in reaction mechanism and procedure.

## 8. IMPORTANT NOTICE IN THE TREATMENTS

### 1. Treatment of assay samples

1. Use serum or plasma samples obtained by ordinary standard method.
2. Turbid samples or those containing insoluble materials should be centrifuged before assay and remove those materials.
3. Measure the samples as soon as possible after sampling.

### 2. Storage of assay samples.

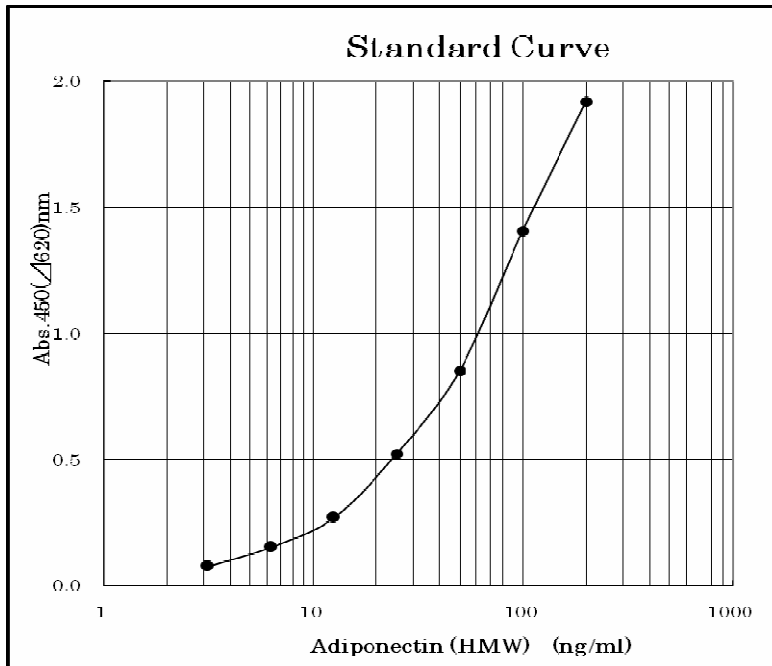
If assay samples have to be stored for a long period, freeze samples and store below  $-35^{\circ}\text{C}$ . Avoid repeated freezing and thawing.

### 3. Influence of interfering substances

If presence of interfering substances is suspected, examine by a dilution test using more than 2 points.

## 9. ASSAY RANGE AND ASSAY VALIDATION

### 1. An example of standard curve



### 2. Specificity

Using two antibodies this kit can assay mouse/rat high molecular weight adiponectin (HMW).

### 3. Precision and reproducibility

1. Within assay variation (2 samples, 5 replicates assay)  
Average C.V. is less than 5%.
2. Reproducibility (3 samples, duplicates assays, 4 days)  
Average C.V. is less than 5%.

### Assay precision (intra assay variation)

Well	Sample A	Sample B
1	29.5	129
2	30.7	125
3	29.8	128
4	29.0	126
5	29.6	126
mean.	29.7	127
SD	0.631	1.89
CV(%)	2.12	1.49

Unit:ng/ml

### Reproducibility (inter-assay variation)

Samples	Day 1	Day 2	Day 3	Day 4	mean.	SD	CV(%)
C	196	192	196	190	193	2.63	1.36
D	126	130	125	125	127	2.27	1.79
E	62.5	59.1	60.7	60.3	60.7	1.41	2.33

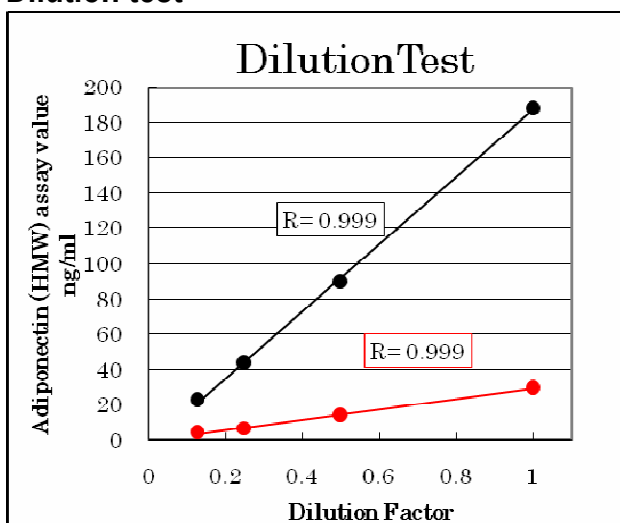
Unit: ng/ml n=2

### Recovery test

Sample No.H				Sample No.I			
Added	Found	Recovered	Recovery (%)	Added	Found	Recovered	Recovery (%)
0.00	68.5	—	—	0.00	23.3	—	—
35.0	103	34.5	98.6	18.0	40.3	17.0	94.4
65.0	132	63.5	97.7	26.0	50.6	27.3	105
95.0	165	96.9	102	32.0	55.5	32.2	101

Unit: ng/ml n=2

## Dilution test



## Cross-reactivity

Species	Substances examined	Reactivity(%)
Mouse	Adiponectin(HMW)	100
	Adiponectin(Hexamer)	< 5
	Adiponectin(Trimer)	-
	Adiponectin(Monomer)	-
	MCH	-
	TNF- $\alpha$	-
	INF $\gamma$	-
	Insulin	-
	Leptin	-
Rat	Adiponectin(HMW)	100
	Adiponectin(Monomer)	-
	TNF- $\alpha$	-
	INF $\gamma$	-
	Insulin	-
	Leptin	-
Human	Adiponectin(HMW)	< 45
	Adiponectin(Trimer)	-
	Adiponectin(Monomer)	-

(Concentration examined at 1000ng/ml)

+: Cross-reacted    -: Not reacted

## 10. INFLUENCE OF INTERFERING SUBSTANCES

Hemoglobin: None up to 80mg/dl

Bilirubin (Free form, bound form): None up to 10mg/dl

Chyle: None up to 1000 formagene turbidity

## 11. STABILITY OF SAMPLE

Freezing and thawing: Stable up to 2 freeze-thaw cycles.

At refrigerator temperature : Stable up to 3 days. Recommend freezing at -20°C.

### **Molecular size specificity of the Mouse/Rat Adiponectin (High Molecular Weight ) ELISA KIT by mouse serum fractionation**

Condition of Fractionation

System: AKTA explorer 100s (Pharmacia Biotech)

Column: HiPrep 26/60 Sephacryl S-300 (Pharmacia Biotech)

Elution: 50mM PBS pH 7.2

Wavelength: 280 nm

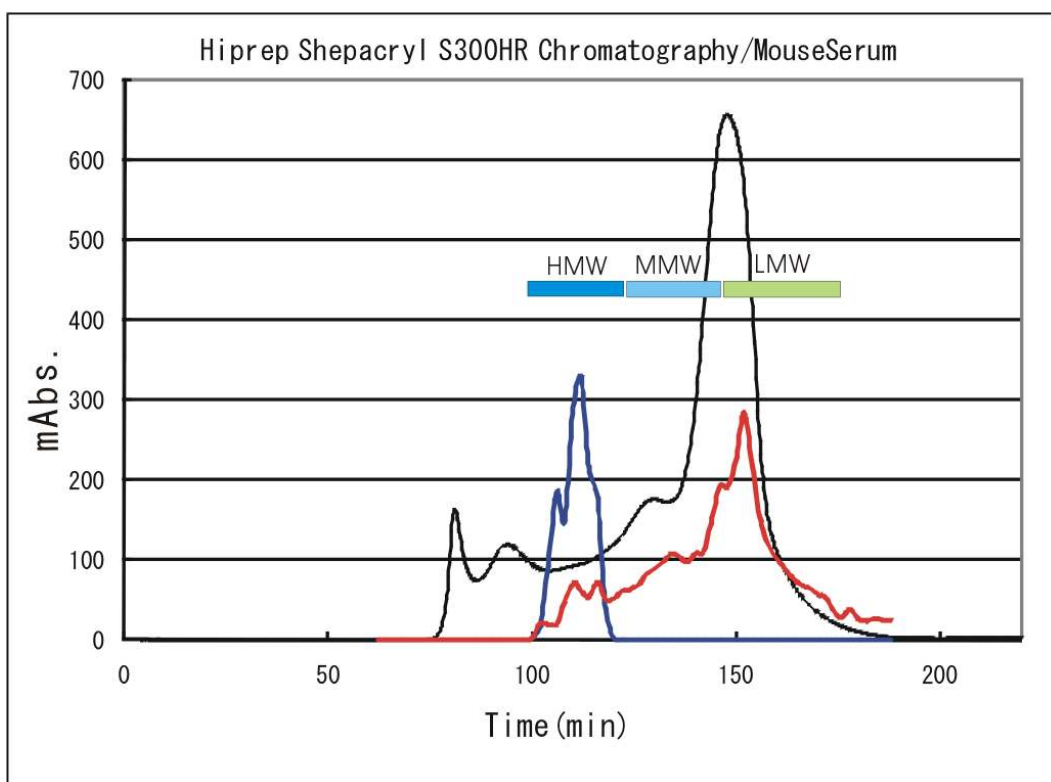
Flow rate: 2ml/min

Sample: Mouse serum: Balb/cAJcl, 6w, male (CLEA Japan)

Loaded volume of the sample: 1.5ml

Absorbance of every fraction was measured at 280nm.

Then each fraction was assayed with Shibayagi's HMW kit (absorbance 450-620nm) and also with commercially available kit for "total adiponectin" (absorbance at 450nm).



It is clearly shown that Shibayagi's kit measures only HMW.

## 12. STATEMENTS AND PRECAUTION

1. The reagents included in this assay kit should be used only for research works.
2. The reagent solutions of the kit should be used principally immediately after reconstitution. Otherwise, keep them in a dark place with the temperature 2-8 °C.
3. The reagents were prepared specifically for each lot in order to give accurate results by their combination within the kit. So, do not combine the reagents in the kit of other lot number. Even if the lot number is the same, do not mix the reagents with those that have been preserved for some period.
4. Pipetting and dilution of the reagent solutions should be made accurately because these steps influence the assay precision.
5. Do not dry the anti-adiponectin-coated plate to avoid denaturation of the coated antibody.
6. Measurement of the reaction time should be started from the pipetting of reagent to the first well.
7. Prepare the standard curve in each assay.
8. Dilution of the assay sample must be carried out using only the buffer solution included in the kit.
9. Storage condition for the kit should be strictly followed.

10. Be careful not to allow the reagent solutions of the kit to touch the skin and mucus. Use special care when handling the Reaction stopper because it is 1M sulfuric acid.
11. Avoid allowing the HRP-conjugated anti-adiponectin solution, chromogenic substrate reagent, and reaction stopper to contact any metal.
12. In treating assay samples of animal origin, be careful for possible biohazards.
13. As the anti-adiponectin-coated plate is module type of 8wells x 12 rows, each row can be separated by a cutter and used independently.

### **13. STORAGE CONDITION**

Store the kit at 2~8 °C. Do not freeze.

### **14. TERM OF VALIDITY**

Six months from production. Expiration date is indicated on the container.

### **15. UNIT OF PACKAGE**

96 wells/1 plat

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