Rat GH (Growth Hormone) ELISA KIT

Research Reagent

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 rat GH with high sensitivity using Sandwich assay principle.

Advantage

(1) Rapid assay (total reaction time:5 hours.).
(2) A small sample volume (5 µl).
(3) An ecologically excellent preservative is used.
(4) Every reagent is provided in liquid form and ready to use.
(5) Excellent precision and reproducibility.

Components

	Reagents	Amounts
(A)	Anti-GH-coated plate	96 wells(8x12) / 1 plate
(B)	Standard rat GH solution (20 ng/ml)	100µl / 1 vial
(C)	Buffer solution	60ml/1 bottle
(D)	Biotin-conjugated anti-GH	100µl/ 1 vial
(E)	HRP-conjugated streptavidin	100µl/ 1 vial
(F)	Chromogenic substrate reagent (TMB)	12ml/ 1 vial
(H)	Reaction stopper (1M H_2SO_4)	12ml/ 1 vial
(I)	Concentrated washing buffer (10x)	100ml/ 1 bottle

Assay samples

Rat serum or plasma :5 µl in the standard procedure.
The volume of assay sample can be applied in the range of 5 ~25µl. In such case, the final volume to be added to the well should be adjusted to 50µl using assay buffer (C) **as sample + buffer = 50µl.**

Assay range

31.3 ~ 2000 pg/ml…This range corresponds to 313 ~ 20000 pg/ml of original serum/plasma level in the standard procedure.

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 (A densitometer for microplate).

2. Preparation of reagents
(1) Washing buffer:Dilute the concentrated washing buffer (I) to 10X with purified water.
(2) Biotin-conjugated anti-GH (D):Dilute to 100X with the buffer solution(C).
(3) HRP-conjugated streptavidin (E):Dilute to 100X with the buffer solution(C).
(4) Other reagents are used as they are.
(5) Use all the reagent solutions after bringing them up to room temperature (20-25C).

3. An example of preparing standard solutions
(An example) Dilute the original standard solution (B) with the buffer solution to prepare 2000pg/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.)

Concentration(pg/ml)	2000	1000	500	250	125	62.5	31.3	0
Standard solution(µl)	50**	200*	200*	200*	200*	200*	200*	0
Buffer solution (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 getting back to room temperature.

(1) Rinse the Anti-GH coated wells (A) by filling the washing buffer and discarding the buffer 3 times. Remove the residual buffer in the wells by striking the plate upside-down onto several sheets of folded paper towel.
(2) Pipette 45µl of buffer solution into the wells for samples, then add 5 µl of sample.
Alternatively, it would be convenient to dilute the assay samples to 10X in test tubes beforehand, and pipette 50µl of the diluted sample to each well.
The sample volume can be applied in the range of 5 ~ 25 µl with buffer volume adjusted to make 50 µl **(Sample + buffer = 50 µl)**.
(3) Pipette 50µl of the standard solution to the assigned wells for preparing a standard curve.
(4) Shake the plate gently on a plate shaker.
(5) Incubate for 2 hours at room temperature (20-25C).
(6) Discard the reaction mixture, and then wash the plate 3 times and remove residual washing buffer in the wells as in (1).
(7) Pipette 50µl of Biotin-conjugated anti-GH solution to all wells. Then shake gently on a plate shaker.
(8) Incubate the plate for 2 hours at room temperature.
(9) Discard the reaction mixture, and then wash the plate 3 times and remove residual washing buffer in the wells as in (1).
(10) Pipette 50µl of HRP-conjugated avidin solution to all wells, and shake gently on a plate shaker.
(11) Incubate for 30 minute at room temperature.
(12) Discard the reaction mixture, and then wash the plate 3 times and remove residual washing buffer in the wells as in (1).
(13) Pipette 50µl of Chromogenic substrate reagent to wells, and shake gently on a plate shaker.
(14) Let the plate stand for 30 minutes at room temperature.
(15) Add 50 µl of the Reaction stopper (H) to all wells and shake.
(16) Measure the absorbance of each well at 450 nm (sub-wave length, 620nm) by a plate reader within 30 minutes.

Summary of Assay Procedure

Anti-GH-coated plate(wells)
↓
Washing 3 times
↓
Buffer 45 µl + Sample 5 µl (diluted sample 50µl)* or Standard 50µl
↓
Shaking, then reaction for 2 hours at 20~25C
↓
Washing 3 times
↓
Biotin-conjugated anti-GH 50µl
↓
Shaking, then reaction for 2 hours at 20~25C
↓
Washing 3 times
↓
HRP-conjugated avidin 50µl
↓
Shaking, then reaction for 30 mins at 20~25C
↓
Washing 3 times
↓
Chromogenic substrate reagent 50µl
↓
Shaking, then reaction for 30 mins. at 20~25C
↓
Reaction stopper 1M H_2SO_4 50µl
↓
Shaking, then measurement of absorbance at 450nm (sub. 620nm)

*Refer to the detailed procedure (2) for sample volume.

Calculation of GH concentration

(1) Prepare a standard curve by plotting absorbance* (Y-axis) against standard concentration (ng/ml) on X-axis. *Absorbance at 450nm minus absorbance at 620nm.
(2) Read GH concentration of a sample from its absorbance*, and multiply the assay value by dilution rate (in the standard procedure, the dilution rate is 10). 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.
* We recommend the use of 3rd order regression curve or 4 parameter method in computer calculation.

Important notice in the treatments

1. Treatment of assay samples

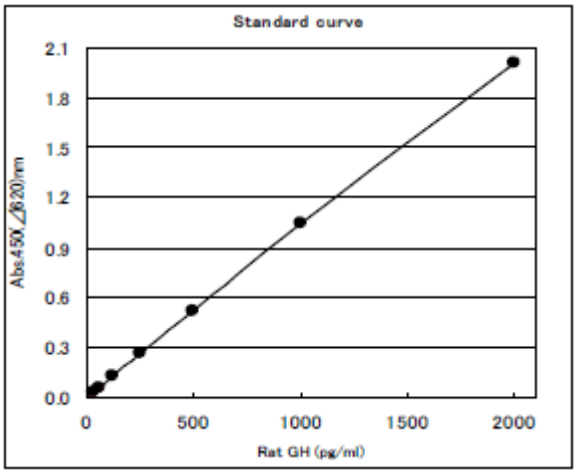
(1) Use serum or plasma samples obtained by ordinary standard method.
(2) Turbid samples or those containing insoluble materials should be centrifuged before assay and remove those materials.
(3) Measure the samples as soon as possible after sampling.

2. Storage of assay samples.
If assay samples have to be stored for a long period, freeze samples and store below –35C. 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.

Assay range and assay validation

1. A model standard curve



2. Specificity
GH specific monoclonal antibodies are used.

3. Precision and reproducibility
(1) Within assay variation (2 samples, 8 replicates assay)
Average C.V. is less than 5%.
(2) Reproducibility (3 samples, 4 replicates assay, 4 days)
Average C.V. is less than 5%.

Notice

1. General caution on kits
(1) Place this kit keeping away from direct sunshine or heat.
(2) Do not freeze the kit.
(3) After opening the containers of reagents, use them as soon as possible. When storing, keep the tightly capped bottles under the temperature of 2~8C in a dark place.
(4) The reagents of the kit must not be used for other purpose than ELISA.
(5) Use fully dried lab-wares (pipette tips, glasswares) in assay.
(6) Temperature should be strictly kept in the range of 20~25C during assay process.

2. Precaution about kit and assay results
(1) Do not use the kit after its expiration date.
(2) The reagents were prepared specifically for each lot in order to give accurate results. 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. Before starting assay, every reagent should be brought up to the room temperature (20~25C). Incomplete temperature condition may influence the assay results and cause incomplete performance of the kit.
(3) Judgment of the assay results should be carefully made from the overall situation including condition of the animals and results of other examinations.

3. Precaution in treatments
(1) In treating assay samples of animal origin, be careful for possible biohazards.
(2) After assay, samples and waists should be dipped in 1% formalin, 2% glutaraldehyde, or more than 0.1% sodium hypochlorite solution for more than 1 hour before discarding them. Alternatively, treat them in autoclave for sterilization.
(3) Be careful not to allow the reagent solutions of the kit to touch the skin and mucus. Take special care when handling the stopping solution because it is 1M sulfuric acid.
(4) If such hazard happens, wash the place with enough volume of water, and if necessary, consult a doctor as soon as possible.

Storage condition

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

Term of validity

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

Unit of package

96-wells/1 plate

Product code

AKRGH-010

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