SCETI

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For research use only

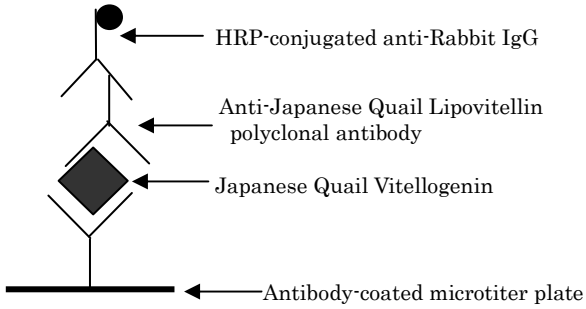
Japanese Quail Vitellogenin（Vg）ELISA Kit

In recent years, influence of Endocrine Disrupting Chemicals (EDC) upon living organisms has been one of the hot topics in the environmental science. However, evaluation methods of chemical compound species that may have EDC effects has not yet been established, and it is absolutely necessary to take action to complete such a task.

Vitellogenin (Vg), a precursor of yolk protein, is a female-specific protein that is present only in the blood of higher oviparous vertebrate organisms. Vitellogenin is normally expressed in the liver under regulation of estrogen (female sex hormone) during an egg formation process, and when it reaches a growing oarian follicle via blood stream it contributes to the egg yolk formation. Since estrogen is known to have an effect upon male species to induce unusual expression of Vg, an ELISA screening method detecting Vg in EDC exposed male animals is the most reliable bio-screening assay system.

〔Principle〕

This kit is based on the ELISA method which uses two different specific anti-Japanese Quail Vg antibodies. Anti-Japanese Quail monoclonal antibody is coated on the microplate, so that Vg in the sample and standard solution is captured. Reacting with anti-Japanese Quail polyclonal antibody and HRP-conjugated anti-Rabbit IgG, the complex of coated-antibody－antigen－antibody is formed. Finally, Vg concentrations are estimated according to the coloring intensity developed with enzymatic reaction.



〔Kit Contents〕

(1)	Antibody-coated microtiter plate (8 wells×12)	1 plate
(2)	The standard, Japanese Quail Vg （200ng/mL）	300μl × 2
(3)	Dilution solution	30mL × 2
(4)	Anti-Japanese Quail Lipovitellin polyclonal antibody (×100)	80μl × 1
(5)	HRP-conjugated anti-Rabbit IgG (×100)	80μl × 1
(6)	OPD (o-phenylendiamine) tablets	2 tab.
(7)	Substrate buffer	15 mL × 1
(8)	Stop solution	6 mL × 1
(9)	Wash solution (×20)	30 mL × 1

〔Required Apparatus〕

(1) A microplate reader
(2) A micropipet
(3) A microplate washer

〔Assay Method〕

(1) Preparation of reagents to be used

① Wash solution
Make sure that the solution does not contain any crystallized material at room temperature. Working solution is prepared by dilution of 30 mL of the stock wash solution with distilled deionized water to 570 mL. This solution is stable for 14 days when stored in a refrigerator.

② Antibody-coated microtiter plate
Add wash solution 300μl to each well and wait another 10 to 60 minutes.

③ Standard dilution
Dilute the concentrated standard solution (200 ng/mL) to make 100、50、25、12.5、6.25、3.13、1.56、0.78 ng/mL with Dilution solution.

④ Anti-Japanese Quail Lipovitellin polyclonal antibody (×100)
Dilute the stock solution of 50μl anti-Japanese Quail Lipovitellin polyclonal antibody with 5 mL of Dilution solution for 96 well reaction. Make solution just before use.

⑤ HRP-conjugated anti-Rabbit IgG (×100)
Dilute the stock solution of 50μl HRP-conjugated anti-Rabbit IgG with 5 mL Dilution solution for 96 well reaction just before use.

⑥ Coloring solution
Dissolve two OPD tablets with 13 mL of Substrate buffer.

(2) Preparation of samples to be used
The serum of Japanese Quail should be diluted more than 8 times with Dilution solution.

(3) Assay procedure
① Discard the wash solution from the wells completely, but do not keep wells in dry condition for reliable data.

② Apply 50μl of each concentration of standard solution and serum samples into the wells and incubate for 1 hour.

③ After the incubation, discard the reaction solution and wash with 300μl wash solution. Repeat this washing procedure another 2 times.

④ Apply 50μl anti-Japanese Quail Lipovitellin polyclonal antibody and incubate for 1 hour.

⑤ After the incubation, wash the wells 3 times with wash solution (See ③).

⑥ Apply 50μl HRP-conjugated anti-Rabbit IgG and incubate for 1 hour.
Substrate buffer should be restored to room temperature from refrigerator at this time.

⑦ After the incubation, wash the wells 3 times with wash solution (See ③).

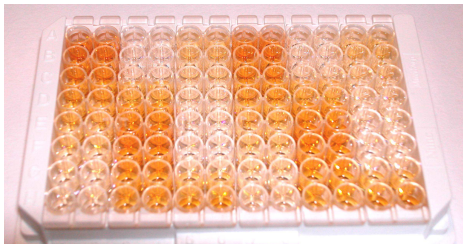
⑧ Apply 100μl Coloring solution into each well for 5 minutes in room temperature.

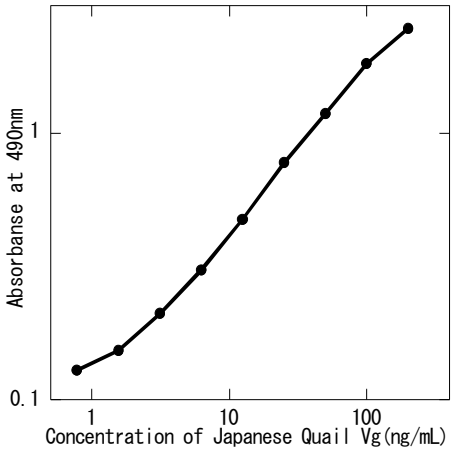
⑨ Apply 50μl of Stop solution for stopping the enzymatic reaction.

⑩ Read absorbance with a microplate reader at 490nm or 492nm.

⑪ Estimate the Japanese Quail Vitellogenin concentration of each sample using the standard curve.

〔Standard curve〕





〔**Reproducibility**〕

Intra- and inter-assay comparison

	N	sample	CV(%)
intra-assay	20	C	4.23
	20	D	5.69
inter-assay	20	C	8.48
	20	D	6.05

Inter-assay comparison of standard curve

	N	Vitellogenin concentration (ng/mL)	CV(%)
intra-assay	7	200	1.16
	7	100	3.23
	7	50.0	0.83
	7	25.0	1.45
	7	12.5	1.02
	7	6.25	1.93
	7	3.13	2.90
	7	1.56	2.14
	7	0.781	2.47
	7	0	2.17

〔**Recovery test**〕

In the recovery study, recoveries 99.8% and 98.3% were obtained for 8 times dilutions of the serum sample.

〔**Usage notes**〕

① The Reagents should be stored at recommended temperature.

② Do not use the reagents which is expired the date of usage.

③ The serum of Japanese Quail should be diluted more than 8 times with Dilution solution.

④ Do not leave the standard, Japanese Quail Vg, for long time under room temperature.

⑤ Since HRP-conjugated anti-Rabbit IgG is not stable under sun light, do not leave it for long time under sun light.

⑥ The glassware for making coloring solution should be clean.

⑦ Since OPD (o-phenylendiamine) is harmful, handle with care.

⑧ Since Stop solution,1N H_2SO_4, is strong acid, handle with care.

⑨ The kit is constructed with well-adjusted combination in each lot. Replaced combination among different lots may cause unexpected results.

⑩ This kit is only for research use. Do not use for medicinal or any other purposees.

⑪ When using the reagents, take care to avoid them from touching to skin, mucous membrane, clothes, and getting into eye.

⑫ If the reagents happen to get into eye or mouth, wash out them and consult a doctor if you need.

⑬ After using the kit, wash your hand very carefully.

⑭ If you find that the packages of the reagents are broken or something wrong, do not use them.

⑮ When you store the reagents, make sure to avoid them from vaporizing, falling down.

⑯ After using the reagents, the packages should be discarded under the established rule.

⑰ We do not guarantee the quality of the packages and accompaniments if not used according this direction.

〔**Storage**〕
-80℃

Supplier

SCETI K.K.
3-6-7 Kasumigaseki, Chiyoda-ku, Tokyo 100-0013, JAPAN
Tel +81(3) 5510-2347 Fax +81(3) 5510-0133
URL: http://www.sceti.jp/export/ e-mail: exp-pet@sceti.co.jp