Datasheet for ABIN579060

Thyroglobulin ELISA Kit

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Overview

Quantity:	96 tests
Target:	Thyroglobulin (TG)
Reactivity:	Rat
Method Type:	Sandwich ELISA
Detection Range:	15.6-1000 pg/mL
Minimum Detection Limit:	15.6 pg/mL
Application:	ELISA

Product Details

Purpose:	This immunoassay kit allows for the in vitro quantitative determination of rat Thyroglobulin, TG concentrations in cell culture supernates, serum, plasma and other biological fluids.
Sample Type:	Cell Culture Supernatant, Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Characteristics:	Rattus norvegicus,Rat,Thyroglobulin,Tg,Tg
Components:	Reagent (Quantity): • Assay plate (1), • Standard (2), • Sample Diluent (1×20 mL), • Assay Diluent A (1×10 mL), • Assay Diluent B (1×10 mL),

Product Details

	• Detection Reagent A (1×120 µL), • Detection Reagent B (1×120 µL), • Wash Buffer(25 x concentrate) (1×30 mL), • Substrate (1×10 mL), • 2 Stop Solution (1×10 mL), • Plate sealer for 96 wells (5), • Instruction (1)
Material not included:	Microplate reader. Pipettes and pipette tips. EP tube Deionized or distilled water.

Target Details

Target:	Thyroglobulin (TG)
Alternative Name:	Tg (TG Products)
Background:	Thyroglobulin is the protein precursor of thyroid hormone and is made by normal well differentiated benign thyroid cells or thyroid cancer cells. Although thyroglobulin levels may be elevated in patients with thyroid cancer, a large number of benign thyroid conditions may also be associated with elevated levels of thyroglobulin, hence an increased thyroglobulin alone in a patient not known to have thyroid cancer is not a sensitive or specific test for the diagnosis of thyroid cancer. Simply examining the thyroid or carrying out a thyroid biopsy can produce significant elevations in the circulating blood level of thyroglobulin. Similarly, patients with thyroid inflammation can have very high levels of thyroglobulin. Patients without a known diagnosis of thyroid cancer do not generally benefit from having the levels of thyroglobulin measured. Some patients with antithyroglobulin antibodies may have inaccurate thyroglobulin levels measured in the lab if the antibodies interfere with the assay, but this is uncommon.
Gene ID:	3185
Pathways:	Thyroid Hormone Synthesis

Application Details

Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	The microtiter plate provided in this kit has been pre-coated with an antibody specific to TG. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for TG and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB

	substrate solution is added to each well. Only those wells that contain TG, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of TG in the samples is then determined by comparing the O.D. of the samples to the standard curve.
Reagent Preparation:	Bring all reagents to room temperature before use. Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer. Standard - Reconstitute the Standard with 1.0 mL of Sample Diluent. This reconstitution produces a stock solution. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions (Making serial dilution in the wells directly is not permitted). The undiluted standard serves as the high standard. The Sample Diluent serves as the zero standard (0 ng/ml).
Assay Procedure:	Allow all reagents to reach room temperature (Please do not dissolve the reagents at 37 °C directly.). All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be resealed and stored at 4 °C until the kits expiry date. Prepare all reagents, working standards and samples as directed in the previous sections. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments. 1. Add 100 µL of Standard, Blank, or Sample per well. Cover with the Plate sealer. Incubate for 2 hours at 37 °C . 2. Remove the liquid of each well, don ' t wash. 3. Add 100 µL of Detection Reagent A working solution to each well. Cover with the Plate sealer. Incubate for 1 hour at 37 °C . Detection Reagent A working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform. 4. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (approximately 400 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. 5. Add 100 µL of Detection Reagent B working solution to each well. Cover with a new Plate sealer. Incubate for 1 hours at 37 °C . 6. Repeat the aspiration/wash as in step 4.

	7. Add 90 µL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate within 30 minutes at 37 °C . Protect from light. 8. Add 50 µL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. 9. Determine the optical density of each well at once, using a microplate reader set to 450 nm. Important Note: 1. Absorbance is a function of the incubation time. Therefore, prior to starting the assay it is recommended that all reagents should be freshly prepared prior to use and all required strip-wells are secured in the microtiter frame. This will ensure equal elapsed time for each pipetting step, without interruption. 2. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. The reconstituted Standards can be used only once. This assay requires pipetting of small volumes. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10 µ l for once pipetting. 3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the 5 strips DRY at any time during the assay. 4. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes. 5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent. 6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. 7. Duplication of all standards and specimens, although not required, is recommended. 8. Substrate Solution is easily contaminated. Please protect it from light.
Calculation of Results:	Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the SAA concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. It is recommended to

Application Details

	use some related software to do this calculation, such as curve expert 13.0. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
Restrictions:	For Research Use only

Handling

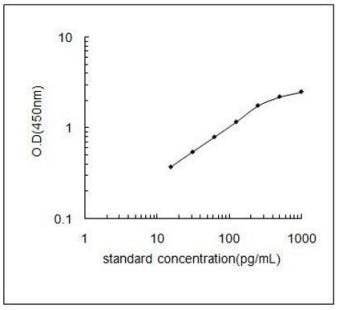
Handling Advice:	.
Storage:	4 °C/-20 °C
Storage Comment:	The Assay Plate, Standard, Detection Reagent A and Detection Reagent B should be stored at -20°C upon being received. After receiving the kit , Substrate should be always stored at 4°C.

Publications

Product cited in:	Kamel, Helmy, Hanafi, Mahmoud, Abo Elfetooh: "Impaired peripheral glucose sensing in F1 offspring of diabetic pregnancy." in: **Journal of physiology and biochemistry**, Vol. 70, Issue 3, pp. 685-99, (2014) (PubMed).

Images

E0355r standard (pg/mL)	OD(450nm)
1000	2.474
500	2.185
250	1.745
125	1.158
62.5	0.792
31.2	0.543
15.6	0.372
0	0.112



ELISA

Image 1.