

ab285258 – Mouse/Rat Thyroxine (T4) ELISA Kit

For quantitative protein detection, establishing normal range etc.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab285258>

Materials Supplied

Item	Quantity	Storage Condition
T4 Ab pre-coated Microplate	8 wells x 12 strips	4°C
T4 Standards	7 x 0.25 mL	4°C
Assay Diluent	12 mL	4°C
T4 Enzyme Conjugate	1.5 mL	4°C
20X Wash Buffer	25 mL	4°C
TMB Substrate	12 mL	4°C
Stop Solution	12 mL	4°C
Controls*	2 x 0.25 mL	4°C

Controls*: Please note that while we still have stock of the previous version of this kit, you may receive kits that do not include the controls.

The controls are included in the most recent version of this kit.

The measured concentrations for the positive controls should fall within the range that is indicated on their respective vials

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

Storage conditions and Reagent Preparation

- Store kit at 2-8°C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes.
- Reagents are stable until the expiration of the kit.
- Do not expose reagent to heat, sun, or strong light.

Wash Buffer: Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26° C).

T4-Enzyme Conjugate Solution: Dilute the T4-enzyme conjugate 1:11 with assay diluent in a suitable container. For example, dilute 160 µl of enzyme conjugate with 1.6 ml of diluent for 16 wells (A slight excess of solution is made). This reagent should be used within 24 hrs for maximum performance of the assay. Store at 2-8°C.

Sample Preparation and Storage

- Collect blood specimens and separate the serum immediately.
- Specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month.
- Avoid multiple freeze-thaw cycles.
- Prior to assay, frozen sera should be completely thawed and mixed well.
- Do not use grossly lipemic specimens.
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

Assay Protocol

Prior to assay, bring reagents, standards and controls to room temperature (18-26°C). Gently mix all reagents before use. The concentrations of standard 1-7 are 0 ug/dL, 1 ug/dL, 2 ug/dL, 5 ug/dL, 10 ug/dL, 15 ug/dL, 25 ug/dL respectively.

Format the microplate wells for each standard, control, and sample to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.

1. Pipette 10 µl of appropriate standard, control, and samples into designated wells.
2. Add 100 µl of diluted T4 enzyme conjugate to all wells. Shake gently for 20-30 sec. to mix.
3. Cover the plate and incubate for 60 min. at room temperature (18-26°C) with shaking.
4. Remove liquid from all wells & wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
5. Add 100 µl of TMB substrate to all wells & incubate for 15 min. at room temperature.
6. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution. 8. Read absorbance on ELISA Reader at 450 nm within 15 min. after adding the stopping solution

Calculation

Construct the standard curve; plot the absorbance for the T4 standards (vertical axis) versus the T4 standard concentrations (horizontal axis). Draw the best curve through the points. Read the absorbance for controls and each unknown sample from the curve.

Record the value for each control or unknown sample.

Example of a Standard Curve

Standard	OD 450nm	Conc. µg/dL
Standard 1	2.657	0
Standard 2	1.392	1
Standard 3	0.842	2
Standard 4	0.431	5
Standard 5	0.192	10
Standard 6	0.132	15
Standard 7	0.07	25

Sensitivity:

The Thyroxine test system procedure has a sensitivity of 0.467 µg/dl. The sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 20(95%certainty) statistics to calculate the minimum dose.

Expected Values

The normal serum/plasma sample is expected to contain between 2.37 µg/dl –9.70 µg/dl T4. It is recommended that each laboratory establish its own range of expected values for the population being tested.

Technical Support

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