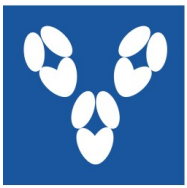

LSBio
an Absolute Biotech Company

Rat fT4 / Free Thyroxine ELISA Kit

(Competitive EIA)

User Manual

Catalog No. LS-F10010

It is important that you read this entire manual carefully before starting your experiment.

This kit is for **Research Use Only**.
This kit is not approved for use in dogs or for clinical diagnosis.

SAMPLE ONLY

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ASSAY SPECIFICATIONS

Target:	Rat fT4 / Free Thyroxine
Specificity:	This kit is for the detection of rat fT4 / Free Thyroxine. No significant cross-reactivity or interference between fT4 / Free Thyroxine and analogues was observed. This claim is limited by existing techniques therefore cross-reactivity may exist with untested analogs.
Sample Types:	This kit is recommended for use with rat serum, and plasma. No other sample types are supported.
Detection:	Colorimetric - 450nm
Measurement:	Quantitative
Detection Range:	4-64 pmol/L
Sensitivity:	Typically less than 1.08 pmol/L
Performance:	Intra-Assay CV=15%; Inter-Assay CV=15%
Limitations:	This kit is for **Research Use Only** and is not intended for diagnostic use. This kit is not approved for use in humans or for clinical diagnosis.

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ASSAY PRINCIPLE

This assay is based on the competition ELISA principle. Each well of the supplied microtiter plate has been pre-coated with a target specific capture antibody. Standards or samples are added to the wells as well as a fixed quantity of biotin-conjugated target antigen. The antigens in the standards or samples compete with the biotin-conjugated antigen to bind to the capture antibody. Unbound antigen is washed away. An Avidin-Horseradish Peroxidase (HRP) conjugate is then added which binds to the biotin. Unbound HRP-conjugate is washed away. A TMB substrate is then added which reacts with the HRP enzyme resulting in color development. A sulfuric acid stop solution is added to terminate color development reaction and then the optical density (OD) of the well is measured at a wavelength of 450 nm ± 2 nm. The OD of an unknown sample can then be compared to an OD standard curve generated using known antigen concentrations in order to determine its antigen concentration. In contrast to typical Sandwich ELISA assays, in competition assay the greater the amount of antigen in the sample, the lower the color development and optical density reading.

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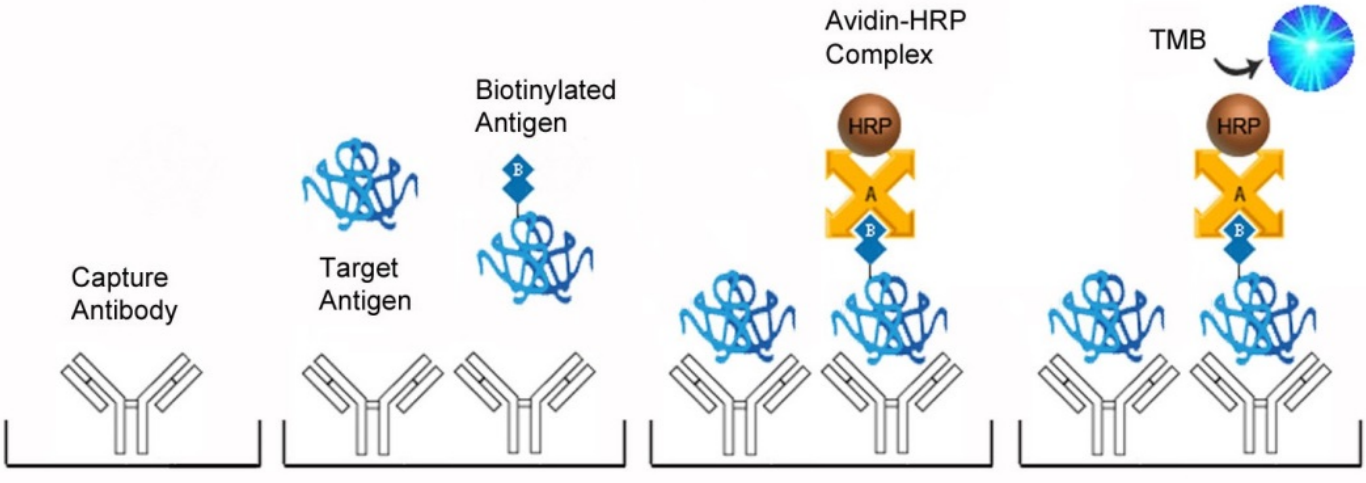
ASSAY PRINCIPLE IMAGE



KIT COMPONENTS AND STORAGE

Component	Quantity
Coated 96-well Strip Plate	1
Standards (S0-S5)	6 vials x 1 ml
Biotinylated Standard	1 vial x 6 ml
HRP-Avidin Conjugate	1 vial x 6 ml
Wash Buffer (20x)	1 vial x 15 ml
Substrate A	1 vial x 7 ml
Substrate B	1 vial x 7 ml
Stop Solution	1 vial x 7 ml
Plate Sealers	4
Instruction Manual	1

Standard Concentrations

Standard	S0	S1	S2	S3	S4	S5
Concentration (pmol/L)	0	4	8	16	32	64

KIT STORAGE

The unopened kit can be stored at 2-8°C through the expiration date. Once opened, the kit can be stored at 2-8°C for 1 month. Unused strips should be kept in a sealed bag with the desiccant provided to minimize exposure to damp air.

OTHER REQUIRED SUPPLIES

- Microplate reader with 450nm wavelength filter, with the correction wavelength set at 600 nm - 630 nm.
- High-precision pipette and sterile pipette tips
- Eppendorf tubes
- 37°C incubator
- Deionized or distilled water
- Absorbent paper

SAMPLE ONLY

SAMPLE COLLECTION

This assay is recommended for use Rat serum and plasma. The following are a collection of sample collection protocols for your reference.

Breast Milk - Centrifuge samples for 20 minutes at 1000×g to remove particulates. Collect the supernatant for assaying. Breast milk often requires a dilution of about 1:500 with PBS*.

Cell Lysates - Collect and pellet the cells by centrifugation and remove the supernatant. Wash the cells 3 times with PBS*then resuspend in PBS*. Lyse the cells by ultrasonication 4 times. Alternatively freeze the cells freeze to -20°C and thaw to room temperature 3 times. Centrifuge at 1500×g for 10 minutes at 2 - 8°C to remove cellular debris. Collect the supernatant for assaying.

Erythrocyte Lysates - Centrifuge whole blood for 20 minutes at 1000×g to pellet the cells and remove the supernatant. Wash the cells 3 times with PBS* then resuspend in PBS*. Freeze (-20°C)/thaw (room temperature) the cells 3 times. Centrifuge at 5,000×g for 10 minutes at 2-8°C to remove cellular debris. Collect the supernatant for assaying. Erythrocyte lysates must be diluted with Standard Dilute before running.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2–8°C within 30 minutes of collection. Collect the supernatant for assaying.

Platelet-Poor Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2–8°C within 30 minutes of collection. It is recommended to centrifuge samples for 10 minutes at 10,000×g for complete platelet removal. Collect the supernatant for assaying.

Sperm and Seminal Plasma – Allow semen to liquefy at room temperature or 37°C. After liquefaction, centrifuge at 2,000×g for 10-15 minutes. Collect seminal plasma supernatant for assaying. Wash the precipitated protein 3 times with PBS* then resuspend in PBS*. Lyse the cells by ultrasonication then centrifuge at 2,000×g for 10-15 minutes. Collect the supernatant for assaying.

Serum - Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for

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20 minutes at approximately 1000×g. Collect the supernatant for assaying.

Tissue Homogenates – Because preparation methods for tissue homogenates vary depending upon tissue type, users should research tissue specific conditions independently. The following is one example only. Rinse tissues in PBS* to remove excess blood and weighed before homogenization. Finely minced tissues and homogenized them in 5-10mL of PBS*with a glass homogenizer on ice. Lyse the cells by ultrasonication or freeze (-20°C)/thaw (room temperature) 3 times. Centrifuge homogenate at 5000×g for 5 minutes. Collect the supernatant for assaying.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter and collect the supernatant for assaying.

Cell culture supernatants, cerebrospinal, follicular, and lung lavage fluids, saliva, sweat, tears, and other biological fluids - Centrifuge samples for 20 minutes at 1000×g to remove particulates. Collect the supernatant for assaying.

* 1xPBS (0.02mol/L pH7.0-7.2)

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SAMPLE COLLECTION NOTES

1. LSBio recommends that samples are used immediately upon preparation. Alternatively samples stored at 2-8°C should be used within 5 days. For long-term storage sample aliquots should be prepared and stored at -20°C if used within 1 month, or -80°C if used within 6 months. Long term storage can result in protein degradation and denaturalization, which may result in inaccurate results.
2. Avoid repeated freeze/thaw cycles for all samples.
3. In the event that a sample type not listed above is intended to be used with the kit, it is recommended that the customer conduct validation experiments in order to be confident in the results.
4. Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results.
5. Influenced by the factors including cell viability, cell number or sampling time, samples from cell culture supernatant may not be detected by the kit.
6. Samples should be brought to room temperature (18-25°C) before performing the assay without the use of extra heating.
7. Sample concentrations should be predicted before being used in the assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
8. LSBio is responsible for the quality and performance of the kit components but is NOT responsible for the performance of customer supplied samples use with the kit.

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REAGENT PREPARATION

Bring all reagents to room temperature (18-25°C) before use.

Wash Buffer: If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved. Prepare 300 ml of Working Wash Buffer by diluting the supplied 15 ml of 20x Wash Buffer Concentrate with 285 ml of deionized or distilled water. Wash Buffer can be stored at 4°C once prepared.

REAGENT PREPARATION NOTES

1. All solutions prepared from concentrates are intended for one-time use. Do not reuse solutions.
2. Reagents may adhere to the tube wall or cap during transport so centrifuge tubes briefly before opening.
3. All solutions should be gently mixed prior to use.
4. To minimize imprecision caused by pipetting, ensure that pipettes are calibrated. Pipetting volumes of less than 10 µL is not recommended.
5. Substrate Solution is easily contaminated so sterility precautions should be taken. Substrate Solution should also be protected it from light.
6. Do not substitute reagents from one kit lot to another. Use only those reagents supplied within this kit.
7. Due to the antigen specificity of the antibodies used in this assay, native or recombinant proteins from other manufacturers may not be detected by this kit.

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ASSAY PROCEDURE

Bring all reagents and samples to room temperature without additional heating and mixed thoroughly by gently swirling before pipetting (avoid foaming). Prepare all reagents, working standards and samples as directed in the previous sections.

1. Set a blank well without any solution.
2. Add 50µl of **Standard** or **Sample** per well. Standards need to be run in duplicate.
3. Immediately add 50µl of **Biotinylated Standard** to each well (not to the blank well), cover with a plate sealer, gently agitate to ensure thorough mixing, and incubate for 60 minutes at 37°C.
4. Aspirate the liquid from each well and wash 3 times. Wash by adding approximately 200 µl of **Wash Buffer** using a squirt bottle, multi-channel pipette, manifold dispenser or automated washer. Allow each wash to sit for 10 seconds before completely aspirating. After the last wash, aspirating remove any remaining Wash Buffer then invert the plate and tap against clean absorbent paper.
5. Add 50µl of **HRP-Avidin Conjugate** to each well (not to blank well), gently agitate to ensure thorough mixing, cover with a plate sealer, and incubate for 30 minutes at 37°C.
6. Aspirate the liquid from each well and wash 3 times as outlined in step 4.
7. Add 50µl of **Substrate A** and 50µl of **Substrate B** to each well (**including** the Blank well), gently agitate to ensure thorough mixing, cover with a plate sealer, and incubate for 15 minutes at 37°C. Protect from light and monitor periodically until optimal color development has been achieved.
8. Add 50µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
9. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm.

SAMPLE ONLY

ASSAY PROCEDURE NOTES

1. **ELISA Plate**: Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be placed in a sealed bag containing desiccant, and stored at 4°C.
2. **Solutions**: 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.
3. **Applying Solutions**: All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible.
4. **Assay Timing**: The interval between adding sample to the first and last wells should be minimized. Delays will increase the incubation time differential between wells, which will significantly affect the experiment's accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples should not exceed 10 minutes.
5. **Incubation**: To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods of time between incubation steps. Do not let wells dry out at any time during the assay. Strictly observe the recommended incubation times and temperatures.
6. **Washing**: Proper washing procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper during the washing process. Do not put absorbent paper directly into the reaction wells.
7. **Controlling Substrate Reaction Time**: After the addition of the Substrates, periodically monitor the color development. Stop color development before the color become too deep by adding Stop Solution. Excessively strong color will result in inaccurate absorbance reading.

SAMPLE ONLY

8. **Reading**: The microplate reader should be preheated and programmed prior to use. Prior to taking OD readings, remove any residual liquid or fingerprints from the underside of the plate and confirm that there are no bubbles in the wells.
9. **Reaction Time Control**: Control reaction time should be strictly followed as outline.
10. **Stop Solution**: The Stop Solution is contains an acid, therefore proper precautions should be taken during its use, such as protection of the eyes, hands, face and clothing.
11. **Mixing**: During incubation times, the use of a micro-oscillator at low frequency is recommended. Sufficient and gentle mixing is particularly important in producing reliable result.
12. Kits from different batches may be a little different in detection range, sensitivity and color developing time. Please perform the experiment exactly according to the supplied instructions.
13. Due to inter- and intra-assay variability, it is recommended that appropriate carry over controls be included between assays.
14. Prior to running valuable samples LSBio recommends that the user runs a preliminary experiment using the supplied controls in order to validate the assay.
15. To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.
16. The kit should not be used beyond the expiration date on the kit label.

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ASSAY PROCEDURE SUMMARY

Prepare all reagents, samples and standards.

Set a Blank well without any solution.

Add 50 µl of **Sample or Standard** to each well.

Add 50 µl of **Biotinylated Standard** to each well (not to the blank well) and incubate for 60 minutes at 37°C.

Aspirate and wash 3 times.

Add 50 µl of **HRP-Avidin Conjugate** to each well (not to the blank well) and incubate for 30 minutes at 37°C.

Aspirate and wash 3 times.

Add 50 µl of **Substrate A** and 50 µl **Substrate B** to each well and incubate in the dark for 15 minutes at 37°C.

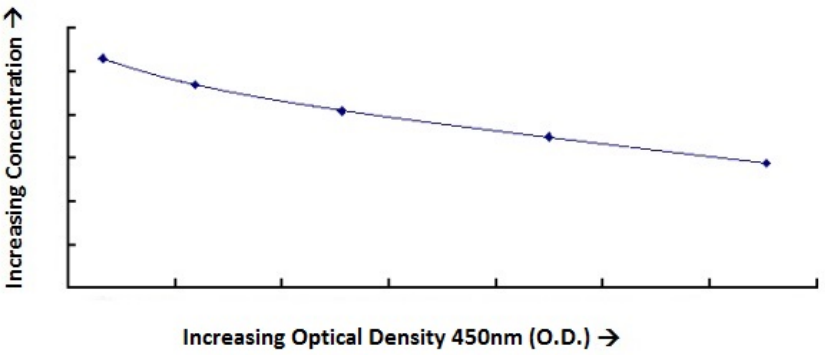
Add 50 µl of **Stop Solution**.

Read within 10 minutes at 450nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample. Create a standard curve by plotting the mean absorbance for each standard on the X-axis against the target antigen 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 target antigen concentration on the Y-axis versus the O.D. of the standards on the X-axis and the best fit line can be determined by regression analysis. The linear equation (Y = mx + b) can be used to calculate the standard curve where Y is the log of the concentration of the standard and x is the O.D. value of the standard. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data: The following standard curve is an example only and should not be used to calculate results for tested samples. A new standard curve must be generated for each set of samples tested.



FOR ONLY

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Poor standard curve	Inaccurate pipetting.	Check pipettes.
	Wells not completely aspirated.	Completely aspirate wells between steps.
Low signal	Too brief incubation times.	Ensure sufficient incubation time.
	Incorrect assay temperature.	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes.	Check pipettes and ensure correct preparation.
	Improper dilution.	
Deep color but low value	Plate reader settings not optimal.	Verify the wavelength and filter setting in the plate reader.
		Open the Plate Reader ahead to pre-heat.

SAMPLE ONLY

Troubleshooting Guide (continued)

Problem	Possible Cause	Solution
Large CV	Inaccurate pipetting.	Check pipettes.
High background	Plate is insufficiently washed.	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer.	Make fresh wash buffer.
Low sensitivity	Improper storage of the ELISA kit.	All the reagents should be stored according to the instructions.
	Stop solution not added.	Stop solution should be added to each well before measurement.

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Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. We recommend briefly centrifuging the vial to dislodge any liquid in the container's cap prior to opening.

Warning: This reagent may contain sodium azide and sulfuric acid. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion. Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.

Returns, Refunds, Cancelations: Any problems with LifeSpan products must be reported to LifeSpan within 10 days of product receipt. The customer must obtain written authorization from LifeSpan before returning items. To request that goods be returned, please contact LifeSpan Technical Support. If an error by LifeSpan BioSciences results in shipment of an incorrect order, LifeSpan will, at its option, either ship a replacement order at no charge, or credit the customer's account for the original product shipped in error. Returns and cancelations may be subject to a 30% restocking fee. Conditions & Warranty: All LifeSpan products are intended for Research Use Only and are not for use in human therapeutic or diagnostic applications. The information supplied with each product is believed to be accurate, but no warranty or guarantee is offered for the products, because the ultimate conditions of use are beyond LifeSpan's control. The information supplied with each product is not to be construed as a recommendation to use this product in violation of any patent, and LifeSpan will not be held responsible for any infringement or other violation that may occur with the use of its products. Under no event will LifeSpan be responsible for any loss of profit or indirect consequential damage, including, but not limited to, personal injuries resulting from use of these products. LifeSpan's liability to any user of Products for damages that do not result from any fault of the user, will be limited to replacement of the Product(s) only, and in no event shall LifeSpan's liability exceed the actual price received by LifeSpan for the Product(s) at issue. LifeSpan shall not be liable for any indirect, special, incidental or consequential damages. LIFESPAN FURTHER DISCLAIMS ANY AND ALL EXPRESS AND IMPLIED OR STATUTORY WARRANTIES WITH RESPECT TO THE PRODUCTS, INCLUDING BUT NOT LIMITED TO ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE. LifeSpan disclaims any and all responsibility for any injury or damage which may be caused by the fault of the user.

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