QUIDEL | Thyretain®

TSI Reporter BioAssay

Frequently Asked Questions

General

What is the sensitivity/specificity of the Thyretain TSI Reporter BioAssay?
The sensitivity was 92% and the specificity was 99% for the Thyretain TSI Reporter BioAssay. The clinical sensitivity and specificity for the device was determined by testing 249 characterized specimens. Additional information regarding the performance characteristics for the clinical studies can be found in the Thyretain TSI Reporter BioAssay Package Insert.

Why is there a lower than expected positive agreement with the TRAb assay?
An explanation for the reduced positive agreement compared to TRAb is the specificity difference between the Thyretain TSI Reporter BioAssay and the comparator device. The comparator device detects autoantibodies to the thyroid stimulating hormone receptors (TSHR), of which there are two classes, stimulating (TSI-hyperthyroidism) and blocking (TBI-hypothyroidism). The comparator device is unable to distinguish between the two antibody types, and the Thyretain TSI Reporter BioAssay detects only stimulating autoantibodies. Thus, during our clinical performance testing, it is likely that some patient sera have TBI and would be positive by the comparator device but negative by the subject device. There currently is no TBI specific cleared device and, as such, it is not possible to further analyze the discordant results.

Is the Thyretain TSI Reporter BioAssay FDA cleared?
Yes. It has been 510(k) cleared. K083391 and K092229.

What detection technology does the Thyretain TSI Reporter BioAssay use?
The Thyretain TSI Reporter BioAssay utilizes a patented bioassay technology to detect TSI in human serum. The CHO Mc4 cells are genetically engineered Chinese hamster ovary cells that express a chimeric form of the human TSHR and a cyclic adenosine monophosphate (cAMP) induced luciferase reporter gene. The cells are cryogenically preserved and provided in measured aliquots. TSI, if present in the patient serum, bind to the chimeric human TSHR on the cell surface. This binding event induces a signaling cascade resulting in increased production of intra-cellular cAMP. This increased production of cAMP is evidenced by increased production of luciferase. The luciferase levels are measured using a luminometer and then compared with the Reference Control.

Specimen Collection, Storage, and Handling

What sample types can I use with the Thyretain TSI Reporter BioAssay?
The system has been cleared for diagnostic use with serum samples only. Use of plasma or whole blood may result in assay failure. The testing of serum that is visibly icteric, hemolytic or lipemic may lead to decreased sensitivity in the detection of TSI.

How should specimens be handled and stored prior to processing?
The specimen should be stored at 2°C to 8°C until it is processed. If the specimen will not be processed within 72 hours, it can be aliquoted and frozen at –20°C for up to 2 months or at –70°C or in a liquid nitrogen Dewar for extended storage beyond 2 months. Samples may go through a maximum of three freeze/thaw cycles. Repeated freezing and thawing of serum samples should be avoided since this may affect specimen biological activity, leading to erroneous results. Samples collected for retrospective analysis should be aliquoted upon receipt and immediately frozen. When testing a frozen sample, it should be thawed rapidly in a 35°C to 37°C water bath for 7-10 minutes similar to processing for Thyretain controls.

Kit Formats

What kit formats are available and what does the kit include?
40-25000.v2 (enough cells for 25 plates)
40-50000.v2 (enough cells for 50 plates)

- CHO Mc4 Fresh Frozen Cells
- Cell Attachment Solution 200 mL
- Growth Medium 200 mL
- Reaction Buffer 500 mL
- Control Set:
  - Positive Control 0.5 mL
  - Reference Control 0.5 mL
  - Normal Control 0.5 mL
- Luciferase Assay Reagent Set:
  - Luciferase Substrate 1 vial
  - Luciferase Assay Buffer Solution 10 mL

Kit Storage

What are the storage conditions?
The following are the components and recommended storage conditions:

CHO Mc4 FreshFrozenCells	Store at –70°C or colder
Control Set (Positive, Reference, and Normal)	
Cell Attachment Solution	Store at 2°C to 30°C
Growth Medium	Store at 2°C to 8°C
Reaction Buffer	
Luciferase Assay Reagent Set	Store at –20°C or colder

NOTE: The CHO Mc4 FreshFrozenCells® must be properly stored (–70°C or below) at all times to maintain optimum performance. The swift transfer of freezer vials to and from the freezer or liquid nitrogen storage is mandatory. Repeated exposure to temperature fluctuations may affect cell viability and/or assay performance.

NOTE: A chest freezer is preferred over an upright freezer. Assay failure is a potential issue due to repeated exposure of the cells to temperature changes over time when an upright freezer is used.

What is the shelf life of the Thyretain TSI Reporter BioAssay and other components?
Individually, the cells and controls have an expiration assigned one year past the date of manufacture; the media expiration is 6 months past the date of manufacture and the luciferase reagents expire 3 years past the manufacture date. When used as intended in the kit format, the shelf-life of the component system is 6 months.

What Thyretain TSI Reporter BioAssay components are available for individual sale?
Luciferase Assay Reagent Set – 40-E2610.v2

CHO Mc4 Fresh Frozen Cells

What are the CHO Mc4 cells?
The CHO Mc4 cells are genetically engineered Chinese hamster ovary cells.

What formats are the CHO Mc4 cells available in?
The CHO Mc4 cells are only available in cryovials containing cryogenically preserved cells in cryoprotective medium containing DMSO.

What are the storage conditions for the CHO Mc4 cells?
The CHO Mc4 cells should be stored at –70**°**C or colder.

What plates are recommended for use with the Thyretain BioAssay?
96 MWP, Black, Clear Bottom (Costar #3603)

Can white plates be used?
The use of white plates will greatly increase the raw RLU values of all specimens and controls. It is recommended that black plates are used in order to retain consistency in the TSI Reporter Assay procedure, as an additional boost in RLU signal is not required.

How should I dilute my cells?
A single vial of CHO Mc4 cells should be rapidly thawed in a 37°C water bath for up to 4 minutes and immediately transferred to 5 mL of pre-warmed growth medium. Improper preparation of the cells may cause erroneous results.

NOTE: The Growth Medium contains the pH indicator phenol red. Repeated exposure of the medium to the air may cause an increase in pH, evidenced by an increasingly deep red color. Limit the exposure of the medium to air as pH levels above 7.9 may affect the assay performance.

How should plates be placed in the incubator?
Uniform heating of the cells is a requirement. The plates are to be placed side-by-side in the incubator rather than stacked. Stacking the plates will cause poor assay performance and greatly increase the risk for both inter- and intra-plate variation as well as assay failure. The plates must be carefully handled in order to avoid uneven distribution of the cells. Use of non-vibrating surfaces is a necessity to ensure uniform distribution of the cells in the wells.

Does the assay require a designated incubator?
No, but it is recommended. Moderate incubator traffic during the cells' growth phase will not affect the performance of the TSI Reporter Assay. However, prolonged, repeated exposure of the cells to temperature and CO_2 fluctuations may lead to reduced overall assay performance. It is strongly recommended that disruption of the cells during growth and induction phase be avoided. Moderate incubator use during the induction period will not cause failure to the TSI Reporter Assay. However, as the ligand-binding process is catalyzed by heat, rapid and prolonged temperature fluctuations during induction are not recommended and may cause assay failure.

NOTE: Extreme care should be taken to ensure that the level of CO2 in the incubator is accurately calibrated to 5%. Prolonged exposure to excessively high (>5.5%) or low (<4.5%) CO2 conditions could affect assay performance.

Luciferase Assay Reagent Set

What temperature do my Luciferase Substrate and Buffer have to be before I can combine them for use?
The Package Insert states that both the Luciferase Assay Reagent Buffer and the Luciferase Substrate must be warmed to room temperature (20°C to 25°C) prior to use. Use of the Substrate and Buffer at other temperatures may cause erroneous results.

NOTE: It is critical to maintain the cell lysis temperature above 20°C. The test result will be affected if the lysis temperature falls to 19°C or less.

How long does my luciferase need to rest after I combine the Substrate and the Buffer?
The Substrate is ready-to-use immediately after it is reconstituted with the Luciferase Assay Reagent Buffer. It is recommended that the vial be gently inverted several times prior to use to ensure complete rehydration of the substrate.

Can I keep my reconstituted Luciferase Substrate for use at a later date?
Yes. You should store the remaining luciferase volume at –20°C for up to 2 weeks and at –70°C or below for up to one month. It should be warmed to room temperature before use.

Quality Control

What is the recommended quality control for the Thyretain TSI Reporter BioAssay?
A reference range is provided with each Control Set which establishes the maximum and minimum acceptable values for the Positive Controls when it is compared to the Reference Control. The positive control range may change with each lot of the Cell and Control set. Please check the target value for the positive control on the positive control reference range label prior to evaluating control validity.

It is required that a positive, reference and negative control be run on each plate that patient samples are tested on. It is good practice to examine the results of the Positive and Normal Controls before examining the test results of the specimens. However different lots might have a different positive control range. Please check the positive control reference range label for the test range before the test. If one or both of the controls fail to perform as expected, review the steps and conditions under which the test was performed to determine the cause(s). Do not report results until controls perform as expected. If the controls do not perform as expected, repeat the run or contact Technical Support before reporting patient results.

Procedural

How should cells be mixed prior to plating?
The cell suspension should be inverted several times before plating. Continual or vigorous vortexing may harm the cells.

What if the plate was not treated with Cell Attachment Solution (CAS) prior to planting the cells?
Not treating the multi-well plates with CAS will cause a decline in the positive SRR and an increase in the negative SRR which can lead to false positives or false negatives.

What if I forgot to decant the Cell Attachment Solution (CAS) before planting the cells?
The RLU values of all samples will drop drastically. This may cause a slight increase in variation (CV%) across the sample duplicate. Additionally, it can cause an increase in both positive and negative sample Specimen to Reference Ratios (SRR) because of the disproportionate decline in reference RLU values.

Should the cells be mixed during plating process?
A lack of thorough mixing of the CHO Mc4 cells while in suspension during this process will cause an eventual decrease in RLU values and increase in variation (CV%) across duplicate samples. This is caused by inconsistent cell dispersion through the suspension due to cell settling. It is recommended that the cells be mixed throughout the plating process by pipetting the solution up and down several times.

What if I observe bubbles in the pipette tip during planting process?
Inconsistent volumes during the plating of the cells can potentially lead to problems with result variation due to cell number issues. It is recommended that air in the tip during planting be avoided. If bubbles appear, replace the tips before proceeding.

What if my cells fail to reach 100% confluence by 15 hours?
If the cells have not reached 90 to 100% confluence in the first 15 hours of growth, allow them to remain in the incubator for an additional 3 hours (maximum of 18-hour growth period). A confluent monolayer is one where cells are in contact with each other forming a continuous sheet of adherent cells on the bottom of the plate well. The confluency of the monolayer is assessed prior to use with a microscope at 100X magnification. If the cells are still under confluent the plate should not be used for patient specimen testing. Contact Technical Support if this is a problem that persists.

Does cell debris in a confluent monolayer effect assay performance?
This occurrence is normal and the cell debris generally is removed upon cell rinsing prior to treatment. Do not attempt to physically remove cell debris as this may disturb the monolayer.

There are loose or floating cells present at the time of use.
The presence of loose, floating, or dividing cells at the time of treatment is only a cause for concern if the overall health of the cells is affected as a result. If the cell monolayer is being pulled away from the plate or disturbed, discard that plate. Piling can occur as a result of cell overgrowth causing dead cells to float. If this is the case, discard that plate.

What if cells are not rinsed with Reaction Buffer?
The RLU values of both the Reference Control and TSI negative serum increase. This could lead to both false negative and false positive results. Care should be taken to ensure that the cells have been rinsed.

What if the cells are not refed with Reaction Buffer and the serum dilutions are added directly to the cells?
This can cause an increase in the RLU signal of the Reference Control and a decrease in the RLU values of TSI containing serum, causing the risk of false negative results. TSI negative serum is generally unaffected.

Additional Information

Where can I find additional information and resources on the Thyretain TSI Reporter BioAssay?
Refer to our website for additional information and resources at quidel.com.

Visit thyretain.com for more detailed information on Graves' disease.

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