CALL FOR PARTNER LABORATORIES

TPO-CATALYZED IODINATION ASSAY

March 2026

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DEADLINE: April 10th, 2026.

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Method: *TPO-CATALYZED IODINATION ASSAY*

Description of the method

1. Summary of the method

Thyroid peroxidase (TPO) is an enzyme located on the apical membrane of thyroid follicular cells. It catalyses the oxidation of iodide using hydrogen peroxide (H_2O_2) and enables the iodination of tyrosyl residues in thyroglobulin (Tg). This process generates monoiodotyrosine (MIT) and diiodotyrosine (DIT). Subsequent coupling reactions produce the thyroid hormones thyroxine (T4) and triiodothyronine (T3).

Certain chemicals can interfere with TPO activity and thereby disrupt thyroid hormone synthesis. The TPO-catalysed iodination assay aims to identify such interference using a biochemical system derived from a human thyroid cell line.

The FTC-238 human follicular thyroid carcinoma cell line has been genetically modified to express recombinant human TPO (FTC-238-hrTPO). Lysates prepared from hematin-stimulated cells contain active TPO and can be used to perform the iodination assay.

In the assay, FTC-238-hrTPO cell lysates are incubated with L-tyrosine, potassium iodide, and hydrogen peroxide in the presence or absence of a test compound. During the reaction, TPO catalyses the conversion of L-tyrosine into monoiodotyrosine (MIT).

MIT formation is quantified using LC-MS/MS and serves as a direct readout of TPO-catalysed iodination.

Primary readout: LC-MS/MS quantification of MIT.

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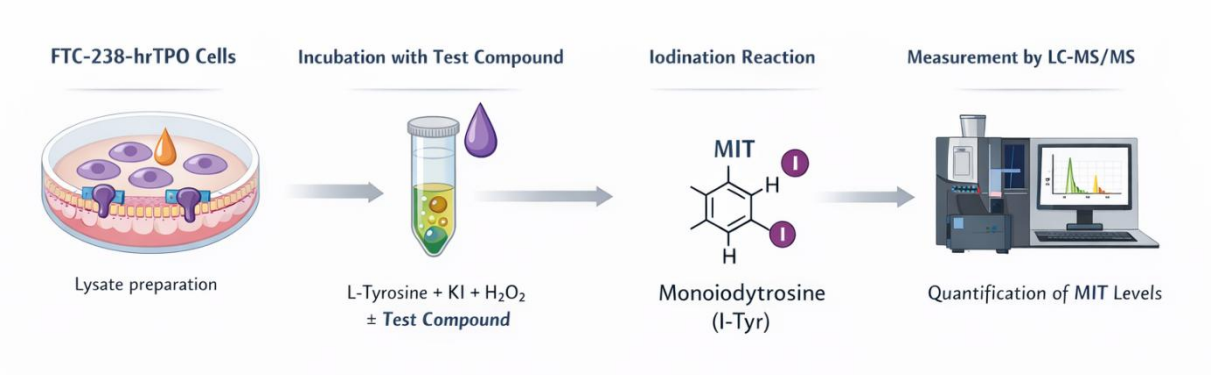

Figure 1: Cultivation of FTC-238-hrTPO cells towards the final test system*. Thyroid peroxidase (TPO) catalyses the oxidation of iodide and the iodination of tyrosyl residues in thyroglobulin, leading to the formation of MIT, DIT, and subsequently the thyroid hormones T3 and T4. To assess potential inhibition of this activity, lysates from FTC-238 cells expressing recombinant human TPO and cultured for 6 weeks are incubated with L-tyrosine, potassium iodide, and H_2O_2 in the presence or absence of a test compound. The formation of monoiodotyrosine (MIT), a direct marker of TPO-catalysed iodination, is quantified by LC-MS/MS.*

Annexes 1 and 2 provides additional information on the necessary equipment, consumables and plate layouts.

2. Experimental Design

2.1 General Considerations

- Solubility testing must be performed for each test chemical.
- A dose range finding experiment should identify the highest soluble concentration.
- Three independent and valid runs are required per chemical.
- A run is considered independent when performed at a different time point or by a different experimenter.
- For a run to be valid, all acceptance criteria must be met.
- Eight concentrations per chemical (C1–C8) are tested in triplicate.
- Two to three chemicals can be tested per plate depending on the experimental layout.

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2.2 Phase I – Transferability

Main assay

The transferability phase aims to demonstrate that laboratories independent from the method developing laboratories can successfully implement the assay.

For each chemical, eight concentrations (C1–C8) will be tested in triplicate. Three independent valid runs (biological replicates) are required.

Four predefined chemicals will be tested:

- A TPO inhibitor (positive control)
- A weak TPO inhibitor (weak positive control)
- A compound without effect on TPO activity (negative control)
- An additional inhibitor of TPO activity

In order to setup and perform the assay a cell lysate will be provided by CRL (lead laboratory) to allow for setup and testing. Phase 1 chemicals are purchased by the labs.

Lysate preparation

In parallel, during this phase labs are required to culture the cells (also provided) and generate their own cell lysate to be used in phase 2. It is also required that the generated lysate is tested and compared to the CRL lysate to ensure quality.

Cells are grown and propagated in up to 40 T175 flasks. Two days before harvest, cells are treated with hematin. On the harvest day cells are centrifuged and lysed in sodium deoxycholate. Lysates are pool and the protein concentration determined (BCA Assay kit).

To determine the amount of lysate and appropriate incubation time for the assay a MIT formation analysis is performed with five lysate concentrations and five timepoint. The MIT formation is determined by the LC-MS/MS method.

The labs own lysate is then compared to the CRL lysate to ensure it can be used in phase 2.

Training

Before any lab work starts the new labs will participate in a practical training session at the lead laboratory (CRL, NL). This will be a two-day event with showcasing and practical testing. Travel and accommodation for this has to be included in the budget (2 persons if possible).

2.3 Phase II – Ring Trial

The ring trial phase evaluates the reproducibility and robustness of the assay across participating laboratories. It aims at testing anonymized chemicals to see if coherent and reproducible outcomes are obtained. Blinded test chemicals are provided by Pepper through a CRO.

Each anonymized chemical will be tested in three independent runs with eight concentrations (C1–C8) tested in triplicate.

A total of up to 25 blinded chemicals will be tested. Test chemicals will be supplied by Pepper. Each participating laboratory will perform solubility testing independently.

3. Deliverables

For each phase a study plan, raw and processed data, and a phase report will be sent to Pepper.

- The study plan should describe and outline the work that will be performed in the specific phase prior to the work starting.
- The phase report should summarize and describe the results and give additional information on learnings, difficulties, and suggestions.

Laboratories are required to participate in regular online meetings with the other laboratories and Pepper.

4. Application to Participate

Labs interested in participating in the validation study should submit an offer by filling in the table below gathering the following information:

Method of interest	*TPO-CATALYZED IODINATION ASSAY*
Date of the proposal	*dd-mm-yyyy*
Laboratory Name and address	
Contact person information [name(s), email(s) and phone number(s)]	
Description of the work	*The way in which the laboratory intends to carry out the work must be described in brief, but sufficient detail to demonstrate the understanding of the project and method.* *The availability of key equipment should be mentioned.*
Starting date and planning	*Expected planning for phase I and phase II, including experimental work as well as reporting.*
Laboratory quality assurance	*QA guaranties of the laboratory must be described (GLP, GIVIMP or other processes).*

Contingency plan (to ensure continuity of the work)	*Indicate how the continuity of work is ensured (e.g. illness, holidays of chief investigator and key persons; response to materials delivery delays…)*
Apply for funding.	*Indicate the financial support you would need on personnel, consumables, and/or training* to participate in the validation as a "test laboratory".* *Provide a separate estimate for each phase (transferability, ring trial).* *Also indicate any in-kind contribution or other sources that could be used as sources for financing this participation.* *Note that Pepper's support is exchangeable between work and consumables.).*

** A 2-3-day training course will be prepared by the developer laboratory to teach and discuss the method in person before starting the transferability phase.*

Offers should be sent to:
tender@ed-pepper.eu

If you have any questions or need clarifications or additional information, please do not hesitate to contact us under tender@ed-pepper.eu.

Deadline for submission: **April 10th, 2026.**

5. References

- Liu and al., In vitro assessment of thyroid peroxidase inhibition by chemical exposure: comparison of cell models and detection methods, In Vitro Systems, 2024, doi: 10.1007/s00204-024-03766-7
- Liu and al., In vitro thyroperoxidase inhibition assessment by LC-ICP-MS-based L-tyrosine iodination assay: comparison with Amplex Ultrared assay and its modifications, In Vitro Systems, 2025, doi: 10.1007/s00204-025-04258-y

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ANNEX 1: Consumables and necessary equipment

Materials and Reagents

1. Biological Material

- FTC-238 human follicular thyroid carcinoma cell line expressing recombinant human thyroid peroxidase (FTC-238-hrTPO)

FTC-238-hrTPO cell lysates prepared from cultured cells (a large number of flasks (approximately 35) will need to be cultured simultaneously in order to obtain a sufficiently large batch of cells and adequate amounts of cell lysate. The material can be frozen and used throughout both phases(TPO lysates will be supplied by the developer laboratory in a ready-to-use state for the setup of the method. During the transferability phase, labs will culture cells and generate their own lysate).

2. Cell Culture Reagents

- Iscove's Modified Dulbecco's Medium (IMDM) with phenol red
- Fetal Bovine Serum (FBS), heat-inactivated
- Geneticin (G418) selection antibiotic
- Penicillin–Streptomycin solution
- Dulbecco's Phosphate Buffered Saline (D-PBS) without calcium and magnesium
- Trypsin-EDTA solution (0.05%)
- Dimethyl sulfoxide (DMSO)
- Hematin solution
- Freezing medium components (culture medium supplemented with FBS and DMSO)

3. Assay Reagents

- L-Tyrosine
- Potassium iodide (KI)
- Hydrogen peroxide (H_2O_2)
- Potassium dihydrogen phosphate (KH_2PO_4)
- Potassium hydrogen phosphate (K_2HPO_4)
- Sodium thiosulfate ($Na_2S_2O_3$)
- Hydrochloric acid (HCl)
- Ultrapure water (Milli-Q or equivalent)
- Potassium phosphate buffer (0.1 M, pH 7.4)
- L-tyrosine stock solution
- Potassium iodide stock solution
- Hydrogen peroxide working solution
- Internal standard working solution containing sodium thiosulfate

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4. Test and Control Items the lab should get access to

- 6-Propyl-2-thiouracil (PTU) – reference inhibitor of thyroid peroxidase
- Bis(2-ethylhexyl) phthalate (DEHP) – negative control compound
- Vehicle solvent (typically DMSO)

5. Protein Quantification Reagents

- Bicinchoninic Acid (BCA) protein assay kit
- Bovine Serum Albumin (BSA) protein standards

6. LC-MS/MS Analytical Reagents

- Monoiodotyrosine (MIT) analytical standard
- 3-iodotyrosine-13C6 (internal standard)
- Methanol (LC-MS grade)
- Acetonitrile (LC-MS grade)
- Formic acid (LC-MS grade)

7. Laboratory Consumables

- Cell culture flasks (e.g., T75 and T175)
- Sterile centrifuge tubes (e.g., 15 mL and 50 mL)
- Microcentrifuge tubes (e.g., 1.5 mL)
- Cryovials for cell storage
- 96-well microplates suitable for plate reader assays
- Amber incubation tubes (U-tubes)
- Glass autosampler vials for LC-MS/MS
- Sterile pipette tips (filtered recommended)
- Disposable serological pipettes

8. Equipment Required for the study that the lab should be equipped with ahead of the call.

- Analytical balance suitable to accurately weigh 5 mg
- Absorbance plate reader for 96-well plates
- Centrifuge with swing-out rotor for 50 mL centrifuge tubes
- Cell culture flasks (75 cm^2 and 175 cm^2)
- CO_2 incubator
- Haemocytometer (e.g., Bürker-Türk counting chamber) or Coulter particle counter
- Inverted light microscope with 4×, 10× and preferably 20× objectives

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- −80 °C freezer for low-temperature storage
- Liquid nitrogen storage container or −150 °C freezer for cryovial storage
- Multichannel pipette
- pH meter
- Pipette controller
- Adjustable pipettes covering volumes from 1 to 5000 µL
- Plate shaker for horizontal shaking
- Safety cabinet or glove box for safe chemical handling
- Sterile laminar flow cabinet (biosafety cabinet)
- Vortex mixer
- Water bath set at 37 °C
- Water bath set at 56 °C
- Ultrasonic water bath
- LC-MS/MS system for MIT quantification

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ANNEX 2: Plating scheme and controls

In concentration-response experiments, each test compound is tested across eight concentrations (C1–C8) in triplicate. In addition, plates also include the control conditions mentioned in the table below:

Description	Acronym used in the fig.2	Replicates
No peroxide control (water instead of H_2O_2)	NP	3
Vehicle control	VC	3
Buffer control (0.1 M potassium phosphate buffer)	No VC	3
Non-enzymatic iodination control using inactivated lysate	NI	3
Reference inhibitor concentration curve	PTU (first plate)	C1–C8 ×3
Reference inhibitor	PTU (other plates)	3
Negative control compound	DEHP	3

Depending on the plate configuration, two (for the first plate of an experimental day) to three (other plates of the experimental day) test chemicals may be included in the same experimental plate (see figure 2).

	Test item 1			Test item 2			PTU		Controls		
C1	C1	C1	C1	C1	C1	C1	C1	C1	DEHP	DEHP	DEHP
C2	C2	C2	C2	C2	C2	C2	C2	C2	VC	VC	VC
C3	C3	C3	C3	C3	C3	C3	C3	C3	No VC	No VC	No VC
C4	C4	C4	C4	C4	C4	C4	C4	C4	NI	NI	NI
C5	C5	C5	C5	C5	C5	C5	C5	C5			
C6	C6	C6	C6	C6	C6	C6	C6	C6			
C7	C7	C7	C7	C7	C7	C7	C7	C7			
C8	C8	C8	C8	C8	C8	C8	C8	C8	NP	NP	NP

	Test item 3			Test item 4			Test item 5		Controls		
C1	C1	C1	C1	C1	C1	C1	C1	C1	DEHP	DEHP	DEHP
C2	C2	C2	C2	C2	C2	C2	C2	C2	VC	VC	VC
C3	C3	C3	C3	C3	C3	C3	C3	C3	No VC	No VC	No VC
C4	C4	C4	C4	C4	C4	C4	C4	C4	NI	NI	NI
C5	C5	C5	C5	C5	C5	C5	C5	C5	PTU	PTU	PTU
C6	C6	C6	C6	C6	C6	C6	C6	C6			
C7	C7	C7	C7	C7	C7	C7	C7	C7			
C8	C8	C8	C8	C8	C8	C8	C8	C8	NP	NP	NP

Figure 2: Plating scheme for Plate 1 (top) and subsequent plates (bottom) used in the concentration–response experiment.