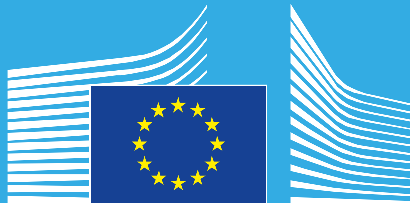
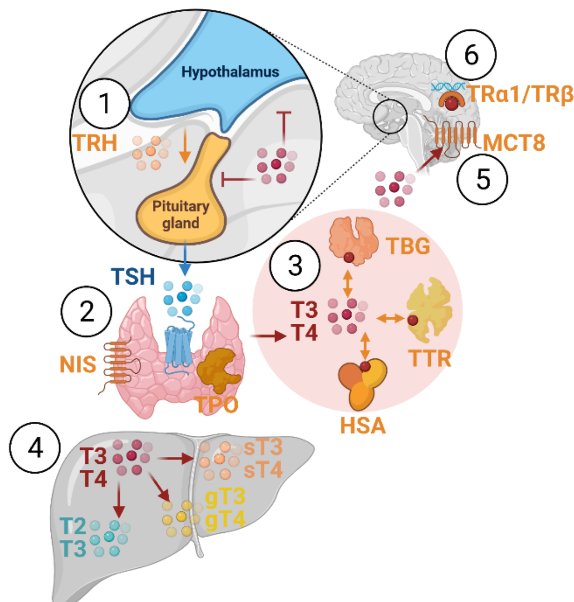


STANDARD OPERATING PROCEDURE

for thyroperoxidase (TPO) extract preparation, used in the TPO inhibition assay based on oxidation of Amplex UltraRed (AUR-TPO), version 1.0

EURL ECVAM validation study of a battery of mechanistic methods relevant for the detection of chemicals that can disrupt the thyroid hormone system

Pedersen E.



2023

Joint Research Centre

This Standard Operating Procedure (SOP) has been prepared within the context of a collaboration agreement with the Joint Research Centre (JRC) Directorate for Health, Consumers and Reference Materials (Chemicals Safety and Alternative Methods Unit F3 / EURL ECVAM), for the validation of mechanistic methods to identify potential modulators of thyroid hormone signalling. It aims to provide evidence-based scientific support to the European policymaking process. The contents of this publication do not necessarily reflect the position or opinion of the European Commission. Neither the European Commission nor any person acting on behalf of the Commission is responsible for the use that might be made of this publication. For information on the methodology and quality underlying the data used in this publication for which the source is neither Eurostat nor other Commission services, users should contact the referenced source. The designations employed and the presentation of material on the maps do not imply the expression of any opinion whatsoever on the part of the European Union concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

This SOP is part of a series of 4 SOPs used to perform the “Thyroperoxidase Inhibition Assay based on Amplex UltraRed (AUR-TPO)”:
SOP “Thyroperoxidase Activity Assay with Amplex UltraRed (AUR-TPO)” v1.0 (used in Part 1 of the validation study)
SOP “Solubility determination by visual inspection” v2.0 (used in Part 1 and Part 2 of the validation study)
SOP “Culture of FTC-238 and FTC-238/hrTPO cells” v3.0 (used in Part 1 and Part 2 of the validation study)
SOP “Thyroperoxidase (TPO) extract preparation” v1.0 (used in Part 1 and Part 2 of the validation study)

SOP “Thyroperoxidase Activity Assay with Amplex UltraRed (AUR-TPO)” has been updated after Part 1, the other SOPs were considered complete to be used also in Part 2.

The method was developed by US EPA and subsequently implemented by the EU-NETVAL test facility RISE (Sweden) within the validation study.

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Thyroperoxidase (TPO) extract preparation

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1 Introduction

1.1 Aim and use of this SOP

The aim of this standard operating procedure is to produce thyroperoxidase (TPO) for use in the AUR-TPO assay (SOP "Thyroperoxidase Activity Assay with Amplex UltraRed (AUR-TPO)", SOP KM 20077). The TPO is prepared as a whole cell extract from cultures of FTC-238 cells (human follicular thyroid carcinoma) transfected with human recombinant TPO (FTC-238/hrTPO). The same extract preparation procedure can also be used with the wildtype FTC-238 cells, in order to provide a background reference for comparison with extracts from the TPO-producing cell line.

This SOP also describes how to measure the protein content of the extracts using the Pierce BCA assay.

1.2 References

Dong H et. Al., A rapid assay of human thyroid peroxidase activity. Toxicology in Vitro, 62, 104662, 2020, https://doi.org/10.1016/j.tiv.2019.104662

Jomaa B et. al., Simple and Rapid In Vitro Assay for Detecting Human Thyroid Peroxidase Disruption, Altex 32(3), 2015. http://dx.doi.org/10.14573/altex.1412201

OECD (2018), Guidance Document on Good In Vitro Method Practices (GIVIMP), OECD Series on Testing and Assessment, No. 286, OECD Publishing, Paris. https://doi.org/10.1787/9789264304796-en

Schmutzler C et. al. The Ultraviolet Filter Benzophenone 2 Interferes with the Thyroid Hormon Axis, 148(6):2835–2844, 2007.

1.3 Cells

The TPO is prepared as a whole cell extract from cultures of FTC-238 cells (human follicular thyroid carcinoma, ECACC 94060902) transfected with human recombinant TPO. The transfected cell line is below referred to as FTC-238/hrTPO, and is described in Schmutzler et. al. 2007. The routine culture of FTC-238 and FTC-238/hrTPO cells is described in a separate SOP (SOP KM 19207).

Cells shall be free from mycoplasma or other contaminations (c.f. SOP KM 11735). Mycoplasma testing shall be performed by DNA extraction followed by real-time PCR method (SOP KM 11732).

2 Waste handling and cleaning

According to the laboratory's internal procedures (SOP KM 11721 and SOP KM 11727). Old cell cultivation vessels and redundant cell suspension must be disposed of as biologically hazardous waste.

3 Equipment and consumables

All equipment should be regularly cleaned, maintained and calibrated, according to the cell culture facility's internal procedures. Both equipment and consumables should be fit for purpose and qualified for use, and their use shall be documented according to the cell culture facility's internal procedures. It is of special importance to verify that no consumables give rise to background noise in the assay where the TPO extract is used, as might happen with many types of plastics in sensitive assays for endocrine disruption. This can be done e.g. by extraction in solvent(s) used in the assay, followed by using the extraction solvent for preparation of VC and blank controls in the assay. For details regarding equipment and software, c.f. SOP KM 11722 and SOP KM 17751, and for consumables SOP KM 11731.

3.1 TPO extract preparation:

Equipment:

- Incubator, 37 °C, humidified, 5 % CO_2 (Inv. No. 900540, 900541 or 901520, or equivalent)
- Centrifuge for pelleting of cells, capable of centrifuging at ~200×g (Inv. No. 900545, KWP01788 or equivalent)
- Refrigerated (4 °C) centrifuge, capable of centrifuging at ~12000×g, preferably centrifuge tubes of size 50 ml (e.g. Inv. No. KWP01788, or equivalent)
- -150°C freezer or equivalent cryostorage for mammalian cells (Inv. No. 900537, or equivalent)
- -80°C freezer (Inv. No. 900538)
- Biological Safety Cabinet class II (Inv. No. 900542)
- Water bath, 37 °C (Inv. No 900546, or equivalent)
- Inverted phase contrast microscope (Inv. No. 900543)
- Precision balance with capacity of 50 g minimum and readability of 0.1 mg (Inv. No 300923, 301021 or equivalent) for weighing of hematin and sodium deoxycholate.
- *Optional:* Cell counter or hemocytometer
- Pipetting aid
- Single channel pipettes capable of dispensing (e.g. 1-10 µl, 10-100µl, 20-200 µl, 100-1000 µl and 500-5000 µl)

Consumables:

- Sterile centrifuge tubes, e.g. 15 ml and 50 ml
- Centrifuge tubes (preferably 50 ml) that can withstand 12000×g
- Tissue culture flasks, e.g. T150 or T300
- Sterile pipette tips
- Serological pipettes 2-50 ml
- pH sticks, capable of measuring pH in the interval 6.0 to 9.0 (for culturing without phenol red)
- Eppendorf tubes for storage of TPO extracts, preferably with low protein binding
- 0.22 µm sterile filter + syringes for sterile filtration of solutions
- Glass storage vial (e.g. 15 ml)

3.2 Protein concentration determination

Equipment:

- 8- or 12-channel pipette capable of dispensing 200µl

- Single channel pipettes (e.g. 10-100µl, 100-1000 µl and 500-5000 µl)
- Plate reader with absorbance measurement function + software (Inv. No. 901171+ 901043, or equivalent), capable of measuring at the wavelength required for the protein measurement assay (562 nm for the Pierce BCA protein assay)
- Shaker, for microtitre plates (e.g., Inv. No. 900535)
- Incubator, 37 °C (e.g. Inv. No. 901520, 900540, or 900541, or equivalent)
- Vortex shaker (e.g. Inv. No. BX51193, or equivalent)

Consumables:

- Transparent 96-well microtitre plates, e.g. TPP cat no 92096 or similar plate (for determination of protein concentration of the TPO extract)
- Centrifuge tube, e.g. 50ml
- Pipette tips
- Serological pipettes 2-50 ml
- Disposable reagent reservoirs
- Polypropylene centrifuge tubes, 15 and 50 ml

4 Medium, chemicals and reagents

For cell culture-related work, ready-to-use sterile solutions shall be used as far as possible, otherwise solutions must be sterilized using a syringe filter (pore size ≤ 0.22µm) or autoclaved (SOP KM 11723). Depending on the stability of the solutions they can be mixed in advance and stored in a fridge/freezer, or should be prepared just before use, see details for each solution/medium. All preparations should be performed using aseptic techniques (see SOP KM 11729). Handling, storage, aliquotation, labeling and documentation of media and other reagents should follow the cell culture facilities internal procedures (c.f. SOP KM 11730 and 11731).

- Complete culture medium 1×FTC, prepared according to the SOP for routine culture of FTC-238 and FTC-238/hrTPO cells (SOP KM 19207).
- TrypLE-EDTA (Gibco Life Technologies cat. no. A12177 and Gibco Life Technologies, cat. no. 15040033, or Gibco Life Technologies cat. no. 12563, or equivalent)
- DPBS without Ca^{2+}, Mg^{2+} (Cytiva Hyclone, cat. no. SH30028.02, or equivalent)
- Hematin (Sigma Aldrich, cat no. H3281or equivalent)
- Sodium deoxycholate, CAS 302-95-4 (Thermo Fisher Scientific Cat # 89904, or equivalent)
- Cell culture grade water (e.g. Cytiva Hyclone, cat. no. SH30529.02, or equivalent)
- *Optional:* Trypan blue stain (Gibco Life Technologies, cat. no. 15250061, or equivalent)
- *Optional:* Penicillin-streptomycin (Cytiva Hyclone, cat. no. SV30010 or equivalent)
- Sodium hydroxide solution to dissolve hematin (Merck KGaA, Cat. No. 1.09959.0001, or equivalent)
- Ice for cold storage of reagents and lysing of cells
- Bovine serum albumin (BSA) standard (if not included in the testing kit; Thermo Scientific cat. no. 23209, or equivalent)
- Pierce™ BCA Protein Assay Kit (Thermo Scientific cat. no. 23225 or 23227, or equivalent)

4.1 Reagent preparation

4.1.1 Hematin solution 0.2 mg/ml (200×)

1. Weigh up the desired amount of hematin, e.g. 10-15 mg in 15ml centrifuge tube.
2. Dissolve to 20 mg/ml in 1 M NaOH
3. Dilute to 1 mg/ml in DPBS, and then sterile filter the solution.
4. Aliquot in suitable amounts (e.g. 2.5 ml aliquots in 15 ml centrifuge tubes). Store at -20°C or -80°C.
5. Dilute to 0.2 mg/ml in DPBS (200× intended use concentration). Store at -20°C or -80°C for up to 6 months.

4.1.2 Sodium deoxycholate 0.1%

1. Weigh up 150-300 mg sodium deoxycholate in 15ml glass storage vial. Store aliquots at <-15 °C.
2. Dissolve in cell culture grade water to 0.1 g/ml (10%).
3. Dilute 100× further in DPBS to 0.1%.

Prepare fresh, protect from light and keep on ice.

5 Culture and stimulation of FTC-238/hrTPO cells

Details regarding routine culture of FTC-238/hrTPO are described in a separate SOP (SOP KM 19207). Before TPO extraction the cells must be stimulated with hematin (one of the building blocks for the enzyme) according to the following:

1. Before harvesting of cells for TPO extraction (section 6.1), stimulate the culture of FTC-238/hrTPO cells with hematin (1µg/ml) for 2-3 days, e.g. for a T300 (50 ml culture volume) add 250µl of a 0.2mg/ml hematin solution (section 4.1.1 above).
2. Incubate the culture at 37°C and 5% CO_2 in a humidified incubator until TPO extraction. To increase the amount of TPO extracted from the culture, it is recommended to aim for a confluency around 85-95% after the incubation period.

The following acceptance criteria must be met before the cells are used for TPO extraction:

- Cell cultures may not be overgrown.
- At harvest cells should have a passage number 2-7 from initiation
- Cells should be free of bacterial, fungal, yeast and mycoplasma contamination.

6 Method

6.1 TPO extraction

TPO from FTC-238/hrTPO cells is prepared as a whole cell extract from cells subcultivated according to SOP "Culture of FTC 238 and FTC 238_hrTPO cells" (SOP KM 19207) and stimulated with hematin (c.f. section 5). The volumes below may be adjusted to obtain a protein concentration of the raw extract in the range 1-2µg/µl. For long-term storage, keep cell lysate at -80°C.

All media and solutions added to the cell culture in step 1-6 below should be at least room tempered (but not higher than 37°C), and preferably pre-warmed to 37°C.

1. Check the culture macroscopically and microscopically for absence of microbial contamination, see SOP KM 11733.
2. Check the medium for absence of contaminations, see SOP KM 11733.
3. Evaluate cell morphology with a phase contrast microscope and record observations according to SOP KM 11734, e.g. if the cells display expected morphology, their degree of confluency, etc. It is recommended to acquire images of the culture.
    - An example of a convenient way of naming acquired microscope images is the following (for easier later identification): 101105_0900_10x_FTC-238_101103-1ee_a.tif
    - The different parts of the name should be interpreted as follows: 101105 = date of acquiring the image, 0900 = time of acquiring the image, 10x = magnification of objective, FTC-238 = cell line, 101103-1ee = Vessel ID, a = identification if more than one image is taken of the same vessel, e.g. a, b, c etc
4. Remove and discard culture medium. Since culturing is performed without phenol red, measure the pH of the culture medium removed from the culture vessel. The pH is normally between 7.0-7.6.
5. Briefly rinse the cell layer with sterile DPBS to remove all traces of serum (details are found in SOP "Culture of FTC 238 and FTC 238_hrTPO cells" (SOP KM 19207).
6. Alternative 1 (scraping of cells)
    i. Scrape the cells and resuspend in a small volume of DPBS (e.g. for a T300: scrape with 5ml DPBS, transfer the cell suspension into a sterile tube, e.g. 15 ml or 50 ml capacity), then rinse the flask again with 5ml extra of DPBS and collect to the sterile tube).
    ii. Spin down the cell pellet at approximately 200×g for 3 minutes.

   Alternative 2 (enzymatic detachment of cells)
    i. Add TrypLE-EDTA 1× solution to flask and incubate at 37°C until the cell layer is dispersed (usually within 5 to 15 minutes). Check that the cell layer is dispersed with the microscope.
       Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach.
    ii. Add complete culture medium (e.g. 5 ml to a T300) to the cell suspension to inactivate the TrypLE. Gently wash any remaining cells from the growth surface of the flask. Use the cell suspension and wash over the surface several times to make sure that most of the cells are collected. Optionally count the cells (to be able to correlate the amount of cells with the amount of extracted protein).
    iii. Transfer the cell suspension into a centrifuge tube (e.g. 15 ml or 50 ml capacity and that can withstand the required centrifugal force) and collect cells by gentle centrifugation (200×g, 3 min). Wash cells twice with DPBS, by dissolving the cell pellet in DPBS (e.g. 10 ml for cells from a T300) followed by gentle centrifugation (200×g, 3 min).
7. Discard the supernatant and lyse the cell pellet in freshly prepared ice-cold 0.1% sodium deoxycholate (DC, in DPBS, see section 4.1.2 above) and incubate on ice for 20 min. 2

mL DC solution is usually sufficient for one T300 flask. Optionally vortex the tube briefly or pipette the solution up and down a few times during the incubation to enhance lysing.

8. Centrifuge the lysed cells for 5 min at 12000×g at 4°C to separate the soluble protein fraction from unlysed cells and debris.
9. Aliquot the supernatant (TPO extract) in, e.g., Eppendorf vials (e.g. ~900µl per vial, except for one vial with ~100µl for protein concentration determination) and store at -80 ˚C until use.
   - Label the vials properly, including date of preparation and name/initials of operator. This is preferably done according to the following format: "TPO _date:XY initials" where X is a running number representing the batch (of that day) and Y a letter (a,b,c, ..) indicating different Eppendorf vials.
   - Record the vials according to the test facility's internal procedures for record keeping of test systems (at RISE GLP cell culture facility this is done in the TPO extract log).
   - Record the work according to the test facility's internal procedures (at RISE GLP cell culture facility this is done in the cell culture study log and TPO extract preparation study log).
   - It is recommended to also freeze down remaining DC solution in suitable aliquots, to be used for the protein concentration determination, for background determination in the AUR-TPO assay, and for diluting the TPO extract to the starting concentration used in the AUR-TPO assay.
10. Measure the protein concentration of the extract according to section 6.2. Typically, the protein concentration is 1-2 µg/µL. Update the TPO extract log with the result.
11. Evaluate the suitability of the batch of TPO extract to be used for the AUR-TPO assay (SOP "Thyroperoxidase Activity Assay with Amplex UltraRed (AUR-TPO)", SOP KM 20077) according to section 6.3.

6.2 Protein concentration determination

The protocol below is adapted/interpreted from the user instructions for the Pierce BCA Protein Assay Kit (Thermo Scientific, cat no 23225/23227) using the albumin standard included with the kit (can also be bought separately). If using a different protein assay kit, the instructions below need to be adapted to the specific kit.

1. Prepare an albumin standard curve according to table 1. Since the 0.1% DC-DPBS solution (section 4.1.2 above), which the TPO extract is prepared in, affects the absorbance measurements, it is recommended to use the same solution as diluent for the standard curve.

Table 1. Preparation of BSA standard curve.

Vial	Volume of Diluent (µL)	Volume and Source of BSA (µL)	Final BSA Concentration (µg/mL)	Amount prepared (µL)
A	0	120 of stock	2000	120
B	50	150 of stock	1500	130
C	130	130 of stock	1000	130
D	70	70 of vial B dilution	750	140
E	130	130 of vial C dilution	500	130
F	130	130 of vial E dilution	250	130
G	130	130 of vial F dilution	125	220
H	160	40 of vial G dilution	25	200
I	160	0	0 = Blank	
Total	**960**			

2. It is recommended to test two different dilutions of the TPO extract (one half the concentration of the other), where both should result in concentrations in the interval 125-1500 µg/ml and OD:s between 0.2 and 2.0. E.g., if the expected protein concentration is in the range 1.5-2.5 µg/µl: Dilute the TPO extract to 50% (with DPBS) and from this dilution prepare a 25% dilution (with 0.05% DC in DPBS). Each extract concentration should be tested in duplicate, and each well requires 25µl of sample.
   - The above procedure ensures that the DC content is the same for both dilutions and roughly the same as in the relevant part of the standard curve.
   - Document the procedures in the "TPO extract preparation study log"
3. Prepare the BCA working reagent (WR) by mixing 50 parts of BCA Reagent A with 1 part of BCA Reagent B (50:1, Reagent A:B). Prepare sufficient volume of WR based on the number of samples to be assayed. For the plate layout in figure 1 with 7 extracts to test, 11 ml is enough.

   NOTE: When Reagent B is first added to Reagent A, turbidity is observed that quickly disappears upon mixing to yield a clear, green WR. The WR is stable for several days when stored in a closed container at room temperature (RT).
4. Pipette 25µL of each standard or unknown sample replicate into a transparent 96-well plate in duplicates, according to Figure 1.

	1	2	3	4	5	6
A	BSA 2000µg/ml		Blank: 0.1% DC-DPBS			
B	BSA 1500µg/ml		TPO extract 1 - 50%		TPO extract 1 - 25%	
C	BSA 1000µg/ml		TPO extract 2 - 50%		TPO extract 2 - 25%	
D	BSA 750µg/ml		TPO extract 3 - 50%		TPO extract 3 - 25%	
E	BSA 500µg/ml		TPO extract 4 - 50%		TPO extract 4 - 25%	
F	BSA 250µg/ml		TPO extract 5 - 50%		TPO extract 5 - 25%	
G	BSA 125µg/ml		TPO extract 6 - 50%		TPO extract 6 - 25%	
H	BSA 25µg/ml		TPO extract 7 - 50%		TPO extract 7 - 25%	

Figure 1. Suggested plate layout for protein measurements

5. Add 200µL of the WR to each well and mix plate thoroughly on a plate shaker for 30 seconds.
6. Cover plate and incubate in the dark at 37°C for 30 minutes.
7. Cool plate to room temperature for ~5 minutes. Measure the absorbance at 562 nm with the plate reader. Use the Gen5 protocol "Pierce_YYMMDD.prt" (use the latest validated version of the protocol, indicated with date in the format YYMMDD). The software will ask for dilution factors, e.g. for 50% and 25% dilutions, the corresponding dilution factors are 2 and 4, respectively.

   Don't forget to:
   - Perform an in-use check of the plate reader according to SOP KM 18988.
   - Label each plate (under plate information).
   - Enter sample IDs.
   - Sign each plate (via the Plate menu).
   - Print the Gen5 report (as a pdf file, then print the pdf).
8. Calculate the protein concentration of the original (undiluted) TPO extract. Gen5 automatically performs the calculations and the results are included in the pdf report.
   - Calculations are performed according to the following: The average 562 nm absorbance measurement of the Blank standard replicates is subtracted from the 562 nm measurements of all other individual standard and unknown sample replicates. A

four-parameter curve (y = (A-D)/(1+(x/C)^B) + D) is fitted to the standard data (mean of duplicates), which is used to determine the protein concentration of each sample.

- Verify that the two measured dilutions are in the required interval of the standard curve (125-1500 µg/ml) and have acceptable OD values (i.e. values that are within a suitable range for the used plate reader), and that the protein concentrations of the original (undiluted) TPO extract estimated from the two dilutions do not differ more than 10% from the total mean. The total mean shall be used as a measure of the protein concentration.

6.3 Qualification of TPO extract for the AUR-TPO assay

Before using a TPO extract for testing of test items with the AUR-TPO assay:

Measure the TPO efficiency for the batch of TPO extract and perform the AUR-TPO assay using the reference item MMI, as described in the SOP for the AUR-TPO assay (SOP "Thyroperoxidase Activity Assay with Amplex UltraRed (AUR-TPO)", SOP KM 20077). For the TPO extract to be qualified for use with the AUR-TPO assay, the following criteria must be met (details are found in section "Acceptance criteria" in the AUR-TPO assay SOP):

- The MMI AC_{50} must pass acceptance criteria.
- The TPO efficiency of the extract must be greater than 3-fold the TPO-free control (BC2).

Document the qualification of the TPO extract in the "TPO extract preparation study log" and in the "TPO extract log".

During the implementation of the test method in the facility, the TPO efficiency of extracts from wildtype cells need to be evaluated in parallel with extracts from TPO-expressing recombinant cells, following the procedure described in the AUR-TPO assay SOP. The TPO efficiency of wildtype cells must be close to 0%.

7 Record keeping and reporting

At a minimum, records should be kept for

- Media and reagents used
- Equipment and consumables used
- Passage history of cells, including observations regarding cell growth, e.g.
    - Confluency estimates.
    - If growth/appearance deviates from expected behaviour.
    - Signs of contamination of cells or media.
- Procedure used for detachment of cells (scraping or enzymatic)
- Documentation of cell counting, when performed
- Documentation of absorbance measurements and subsequent calculations to determine protein concentration
- Documentation of TPO extract qualification for use in the AUR-TPO assay

As soon as possible upon finishing TPO extract preparation, ensure that all documents and raw data are complete, then print, sign (see below), and transfer the following documents generated during the test for pre-archival (these cover the points above):

- Gen5 report from protein concentration determination
- Gen5 System Test Report (from the in-use check of the plate reader)
- Filled out templates:
  - TPO extract preparation study log
    - This Microsoft Excel template is used to document the preparation of TPO extracts and the subsequent protein concentration determination
  - Cell culture study log
    - This Microsoft Excel template is used to document all details regarding the cell culture work performed during the study. The template shall be used for the culture, stimulation and harvest of FTC-238/hrTPO cells for TPO extraction.
  - Cell counting template
    - This Microsoft Excel template serves as an aid for calculating the cell density of a suspension
  - Instrument in-use checks study log
    - This Microsoft Excel template is used to record all performed instrument in-use checks (c.f SOP KM 11722 and SOP KM 19207 for details)
  - Cell culture plastic ware study log
    - This Microsoft Excel template is used to document cell culture plastic ware used during the study. The information specific for the actual study is obtained from the log “Cell culture chemicals and plastic ware log”
  - Chemicals study log
    - This Microsoft Excel template is used to record details related to chemicals, including aliquots and mixing details. The information specific for the actual study is obtained from the log “Cell culture chemicals and plastic ware log”
- Images acquired during culture/harvest

In addition, the vials with TPO extract shall be documented in the “TPO extract log”.

All documents shall be signed and archived according to SOP KM 11995.

All deviations from the Study plan, SOPs or expected behavior shall be reported to the Study Director (via the Principal Investigator, in the case of a multi-side study) immediately, using the “GLP Deviation Reporting form”. The Study Director (in agreement with the Principal Investigator, in the case of a multi-side study) determines whether or not the deviation has affected the final result. If the final result is considered to be affected by the deviation, or if the test does not pass the acceptance criteria or if data is lost or incomplete, the study is considered as failed. The form “Report of failed study” shall then be filled out, signed and archived together with all other documents normally archived (SOP KM 11995), in the cases where these are available.

8 Revision history of SOP

Edition*	Date	Author	Short information about the changes

* Edition of the SOP which is subject to revision. Once the changes are approved the SOP will obtain a new (higher) edition number.

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— via the following form: european-union.europa.eu/contact-eu/write-us_en.

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