# Rat Thrombomodulin (TM) ELISA Kit

## Cat #: orb1946875 (manual)

*For research use only. Not intended for diagnostic use.*

## Product Features

### Intended Use

This ELISA kit applies to the in vitro quantitative determination of Rat TM concentrations in serum, plasma and other biological fluids.

| | |
|---|---|
| Sensitivity | 56.25 pg/mL |
| Detection Range | 93.75-6000pg/mL |
| Specificity | This kit recognizes Rat TM in samples. No significant cross-reactivity or interference between Rat TM and analogs was observed. |
| Repeatability | Coefficient of variation is ≤ 10% |

**Internal Test Data: N/A**

## Assay Principle

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Rat TM. Samples (or Standards) are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Rat TM and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Rat TM, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of Rat TM. You can calculate the concentration of Rat TM in the samples by comparing the OD of the samples to the standard curve.

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## Kit Components

An unopened kit can be stored at 2-8℃ for 6 months. If the opened kit is not used up, store the items separately according to the following conditions.

| Item | Specifications | Storage |
|---|---|---|
| Micro ELISA Plate (Dismountable) | 96T: 8 wells ×12 strips<br>48T: 8 wells ×6 strips | -20℃, 6 months |
| Reference Standard | 96T: 2 vials<br>48T: 1 vial | |
| Concentrated Biotinylated Detection Ab (100×) | 96T: 1 vial, 120µL<br>48T: 1 vial, 60µL | |
| Concentrated HRP Conjugate (100×) | 96T: 1 vial, 120µL<br>48T: 1 vial, 60µL | -20℃, 6 months |
| Reference Standard & Sample Diluent | 1 vial, 20 mL | 2-8℃, 6 months |
| Biotinylated Detection Ab Diluent | 1 vial, 13 mL | |
| HRP Conjugate Diluent | 1 vial, 13mL | |
| Concentrated Wash Buffer (25×) | 1 vial, 30 mL | |
| Substrate Reagent | 1 vial, 10 mL | 2-8℃<br>(Protect from light) |
| Stop Solution | 1 vial, 10 mL | 2-8℃, 6 months |
| Plate Sealer | 5 pieces | RT |
| Product Description | 1 copy | |

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution. The volume of reagents in partial shipments is a little more than the volume marked on the label, please use accurate measuring equipment instead of directly pouring into the vial(s).

**■ Note for Kit**

1. Please wear lab coats, goggles and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories, especially when detecting blood samples or other bodily fluids.

2. A freshly opened ELISA plate may appear a water like substance, which is normal and will not have any impact on the experimental results. Return the unused wells to the foil pouch and store according to the conditions suggested in the above table.

3. Do not reuse the reconstituted standard, biotinylated detection Ab working solution, concentrated HRP conjugate working solution. The unspent undiluted concentrated biotinylated detection Ab (100×) and other stock solutions should be stored according to the storage conditions in the table.

4. Do not use expired reagents. If using the kit in multiple sessions, prepare each component according to the required amount to avoid waste and ensure proper use in subsequent experiments. It is recommended to keep the microplate frame, return any unused wells to the aluminum foil bag, and ensure that all components are used within their shelf life.

5. Do not mix or substitute reagents from other batches or sources with those provided in this kit.

6. Do not reuse diluted standards, biotinylated antibody working solutions, or

enzyme conjugate working solutions. The highest concentration standard working solution can be stored at -20°C for up to two weeks but should not be freeze-thawed repeatedly.

7. It is recommended that all samples and standards be assayed in duplicate.

## Materials Required but Not Supplied

1. Microplate reader with 450 nm wavelength filter (preheat it for 15 min before OD measurement)
2. High-precision transfer pipette, EP tubes and disposable pipette tips
3. Incubator capable of maintaining 37℃
4. Deionized or distilled water
5. Absorbent paper
6. Loading slot

## Sample Preparation

### Sample Collection

**1. Serum:** Allow samples to clot for 1 hour at room temperature or overnight at 2-8℃ before centrifugation for 20 min at 1000×g at 2-8℃. Collect the supernatant to carry out the assay.

**2. Plasma:** Collect plasma using EDTA-$Na_2$ as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2-8℃ within 30 min of collection. Collect the supernatant to carry out the assay.

**3. Cell culture supernatant:** Centrifuge samples for 20 min at 1000×g at 2-8℃. Collect the supernatant to carry out the assay.

### Sample Testing Protocol

1. Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.
2. We are only responsible for the kit itself, but not for the samples consumed during the assay. The users should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
3. The detection range of the kit is not equivalent to the concentration range of the analyte in the sample. It is recommended to consult reference, conduct preliminary experiments, or seek technical support to estimate the concentration of the analyte in your sample. If the analyte concentration in the sample is too high or too low, appropriate dilution or concentration of the sample should be performed.
4. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

### ■ Note for Sample

1. The blood collection tubes should be single-use, pyrogen-free, and endotoxin-free. Whole blood samples should not be directly frozen; they must be processed into serum or plasma for testing or storage.
2. For ELISA experiments, common anticoagulants such as sodium and potassium EDTA, and heparin salts can be used to collect plasma samples. However, for other experiments, such as biochemical assays, the researcher should assess the impact of the anticoagulants.

3. Samples should be assayed within 7 days when stored at 2-8℃, otherwise samples must be divided up and stored at -20℃ (≤1 month) or -80℃ (≤3 months). Avoid repeated freeze thaw cycles. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates.

4. Do not use lysis buffers containing surfactants (such as SDS) or organic reagents (such as methanol) to prepare tissue homogenates, cell lysates, or other types of samples.

5. Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.

## Reagent Preparation

1. Bring all reagents to room temperature (18-25℃) before If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment and store the remaining strips and reagents at required condition.

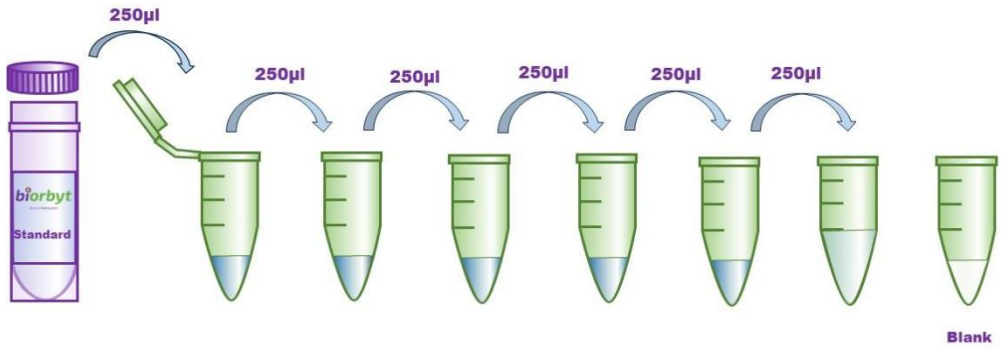
2. Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40 ℃ water bath and mix it gently until the crystals have completely dissolved.

3. Standard working solution:

① Centrifuge: Centrifuge the standard at 10, 000×g for 1 min.

② Add 1mL of Reference Standard &Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 6000pg/mL.

③ Serial dilution: Take 7 EP tubes, adding 250 μL of reference standard & sample dilution buffer to each tube (The volume can be adjusted based on actual usage, e.g., 500 μL/ tube). Transfer 250 μL of the 6000pg/mL standard working solution into the first tube and mix thoroughly to obtain the 3000pg/mL standard working solution. Continue the dilution step by step until the second-to-last tube. The last tube will serve as the blank, and no solution should be transferred from the second-to-last tube. The standard working solution should be freshly prepared and used immediately.




| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 6000 | 3000 | 1500 | 750 | 375 | 187.5 | 93.75 | 0 |
| pg/mL | pg/mL | pg/mL | pg/mL | pg/mL | pg/mL | pg/mL | pg/mL |

4. Biotinylated Detection Ab working solution: Calculate the required amount before the experiment (100μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the

Concentrated Biotinylated Detection Ab at 800×g for 1 min, then dilute the 100× Concentrated Biotinylated Detection Ab to 1× working solution with Biotinylated Detection Ab Diluent (Concentrated Biotinylated Detection Ab: Biotinylated Detection Ab Diluent= 1: 99).

5. HRP Conjugate working solution: Calculate the required amount before the experiment (100µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate at 800×g for 1 min, then dilute the 100× Concentrated HRP Conjugate to 1× working solution with HRP Conjugate Diluent (Concentrated HRP Conjugate: HRP Conjugate Diluent= 1: 99).

6. Experimental Operation Tips

① Solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.

② Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.

③ After adding the Substrate Reagent. The reaction time can be shortened or extended according to the actual color change, but not more than 30 min.

④ Adding the stop solution should be done in the same order as the substrate solution.

## Assay Procedure

1. Determine wells for diluted standard, blank and sample. Add 100µL each dilution of standard, blank and sample into the appropriate wells.

2. Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37℃. Decant the solution from each well and pat it dry against clean absorbent paper.

3. Add 300µL of wash buffer to each well. Soak for 0.5 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times.

4. Add 100µL of Biotinylated Detection Ab working solution to each well. Cover the plate with a new sealer. Incubate for 1 hour at 37°C.

5. Decant the solution from each well, repeat the wash process for 3 times as conducted in step 3.

6. Add 100 µ L of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 30 min at 37℃.

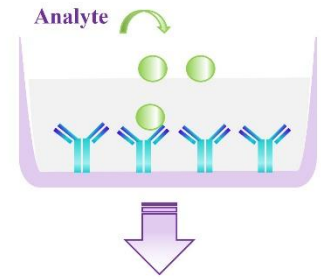
7. Decant the solution from each well, repeat the wash process for 3 times as conducted in step 3.

8. Add 100µL of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37°C. Protect the plate from light.

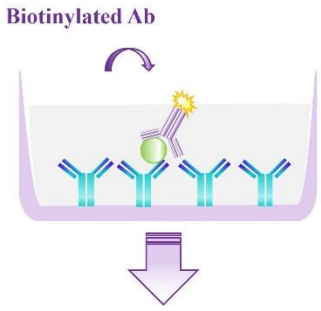
9. Add 50µL of Stop Solution to each well.

10. Determine the Optical Density of each well at once with a micro plate reader set to 450 nm.

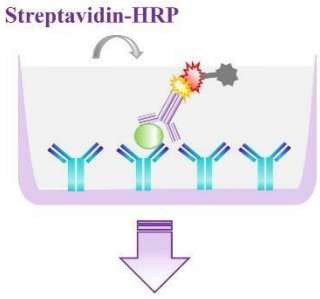
## Assay Procedure Summary



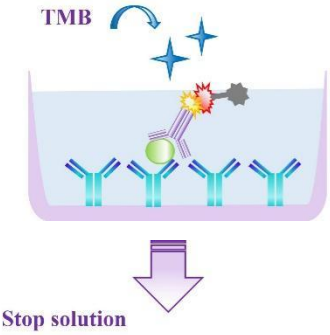

1. Add 100 µL standard or sample to the wells. Incubate for 90 min at 37°C. Aspirate and wash plate for 3 times.



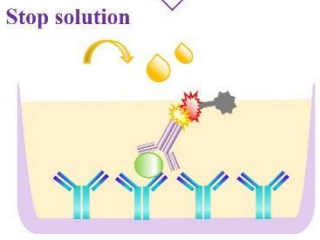

2. Immediately add 100 µL Biotinylated Detection Ab working solution to each well. Incubate for 60 min at 37 °C. Aspirate and wash the plate for 3 times.




3. Add 100 µL Streptavidin-HRP Working Solution to each well, incubate for 30 minutes at 37 °C.




4. Aspirate and wash the plate for 3 times. Add 100 µL Substrate Reagent to each well, incubate for 15 minutes at 37 °C.




5. Add 50 µL Stop Solution to each well. Read the plate at 450 nm immediately, calculation of the results.

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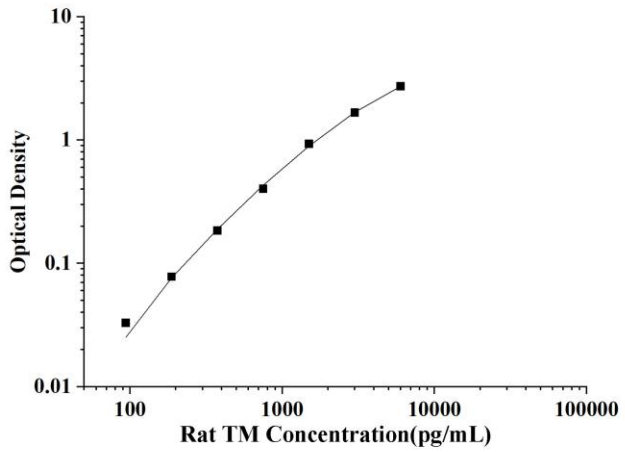
## Calculation of Results

1. The reference standard curve in this manual is for guidance only. The experimenter should use the standard data from each experiment to construct the standard curve and calculate the concentration of the target substance in the sample.

2. When constructing the standard curve, first calculate the average OD values of the standards and sample replicates, and subtract the OD value of the blank well as the correction value. Then, plot the concentration on the X-axis and the corrected OD values on the Y-axis, and use software to perform a nonlinear four-parameter logistic (4PL) fit. This will provide the standard curve, from which the sample concentration can be calculated.

3. The actual concentration is the calculated concentration multiplied by the dilution factor.

| pg/mL | Corrected OD | Standard Curve |
|---|---|---|
| 6000 | 2.731 |  |
| 3000 | 1.669 | |
| 1500 | 0.932 | |
| 750 | 0.403 | |
| 375 | 0.185 | |
| 187.5 | 0.078 | |
| 93.75 | 0.0329 | |
| 0 | 0 | |



## Performance

**Specificity**

This kit is designed for the detection of Rat TM. It has been tested and shows no significant cross-reactivity with other similar substances. However, due to technical limitations and the variability of sample sources, it is not possible to test all related or similar substances for cross-reactivity. Therefore, this kit may potentially exhibit cross-reactivity with other substances that have not been tested.

**Sensitivity**

The minimum detectable Rat TM is 56.25 pg/mL. Sensitivity is determined by calculating the average OD value of 20 blank wells plus 3 times the standard deviation, then converting it to the corresponding concentration.

**Repeatability**

The precision of this kit is ⩽10%, meeting the precision quality control standard. Precision is divided into intra-plate precision and inter-plate precision. It is evaluated by the coefficient of variation (CV) of sample values measured using the same batch and different batches of the kit.

CV (%) = Standard Deviation (SD) / Mean × 100.

**Recovery**

The recovery rate of this kit is 80-120%, meeting the recovery rate quality control standard. The recovery

rate is divided into spiked recovery and sample dilution linear recovery.
Spiked Recovery: Known concentrations of Rat TM are added to different samples for recovery experiments, resulting in the recovery rate range and average value.
Sample Dilution Linear Recovery: Samples containing Rat TM are diluted at different ratios (e.g., 2x, 4x, etc.), and the recovery rate range and average value are determined.

**Stability**

It has been determined that when stored according to the instructions, the kit performs best within its expiration date.

## ELISA Troubleshooting

**High background/non-specific staining**

| Description of results | Possible reason | Recommendations and precautions |
|---|---|---|
| After termination, the whole plate results show a uniform yellow or light color; or the Standard curve is linear but the background is too high | The yellowing of the whole plate may be caused by wrong addition of other reagents | Check the components and lot numbers of the reagents before the experiment, and confirm that all components belong to the corresponding kit. Reagents from different kits or different lot numbers cannot be mixed. |
| | ELISA plate was not washed sufficiently | Make sure that the same amount of Washing Solution is added to each microwell during the washing process. After washing, press the ELISA plate firmly on the absorbent paper to remove the residual buffer. |
| | Incubation time too long | Please strictly follow the steps of the manual |
| | Streptavidin-HRP contaminates the tip and TMB container or positive control contaminates the Pre-coated Microplate | When absorbing different reagents, the tips should be replaced. When configuring different reagent components, different storage vessels should be used. Please use a pipette during operation. |
| | Biotinylated Antibody or Streptavidin-HRP concentration too high | Check whether the concentration calculation is correct or use after further dilution. |
| | Substrate exposure or contamination prior to use | Store in the dark at all times before adding substrate. |
| | Color development time is too long | Please strictly follow the steps of the manual. |
| | The wrong filter was used when the absorbance value was read | When TMB is used as the substrate, the absorbance should be read at 450 nm. |

**NO color plates**

| Description of results | Possible reason | Recommendations and precautions |
|---|---|---|

| | | |
|---|---|---|
| After the color development step, all wells of the ELISA plate are colorless; the positive control is not obvious | Mixed use of component reagents | Please read labels clearly when preparing or using |
| | In the process of plate washing and sample enzyme contaminated addition, the marker is and inactivated, and loses its ability to catalyze the color developing agent | Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as $NaN_3$, etc.) and confirm that the container for preparing the Wash Solution has been washed. |
| | Missing a reagent or a step | Review the manual in detail and strictly follow the operating steps |

**Light color**

| Description of results | Possible reason | Recommendations and precautions |
|---|---|---|
| The Standard is normal, the color of the sample is light | The sample uses $NaN_3$ preservative, which inhibits the reaction of the enzyme | Samples cannot use $NaN_3$ |
| | The sample to be tested may not contain strong positive samples, so the result may be normal | In case of doubt, please test again. |
| The visual result is normal, but the reading value of the microplate reader is low | Wrong filter used for absorbance reading | When TMB is used as the substrate, the absorbance should be read at 450 nm. |
| All wells, including Standard and Samples, are lighter in color | Insufficient incubation time | Timer accurate timing |
| | Insufficient color reaction | Usually 15 - 30 minutes |
| | The number of washings increases, and the dilution ratio of the concentrated lotion does not meet the requirements | Reduce the impact of washing, dilute the concentrated lotion and washing time according to the manual, and accurately record the washing times and dosage. |
| | Distilled water quality problem | The prepared lotion must be tested to see if the pH value is neutral. |
| | In the process of plate washing and sample addition, the enzyme marker is contaminated and inactivated, and loses its ability to catalyze the color developing agent. | Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as $NaN_3$, etc.), confirm that the container for preparing the Washing Solution has been washed, and confirm that the purified water for preparing the Washing Solution meets the requirements and is not contaminated. |

| | | |
|---|---|---|
| | The kit has expired or been improperly stored | Please use it within the expiration and store it in accordance with the storage conditions recommended in the manual to avoid contamination. |
| | Reagents and samples are not equilibrated before use | All reagents and samples should be equilibrated at room temperature for about 30 minutes. |
| | Insufficient suction volume of the pipette, too fast discharge of pipetting suction, too much liquid hanging on the inner wall of the tip or the inner wall is not clean. | To calibrate the pipette, the tips should be matched, each time the tips should fit tightly, the pipetting should not be too fast, and the discharge should be complete. The inner wall of the tips should be clean, and it is best to use it once. |
| Poor repeatability | Incubation temperature constant temperature effect is not good | Keep the temperature constant to avoid the local temperature being too high or too low |
| | When adding liquid, too much remains on the medial wall of wells | When adding liquid, the tip should try to add liquid along the bottom of the medial wall of wells without touching the bottom of the hole. |
| | Reuse of consumables | The tips should be replaced when different reagents are drawn, and different storage vessels should be used when configuring different reagent components. |
| | The bottom of the microwell is scratched or there is dirt | Be careful when operating, be careful not to touch the bottom and wipe the bottom of the microplate to remove dirt or fingerprints. |
| | | Technical repetition of the same sample for 3 times, including more than 2 approximate values. |
| | Cross-contamination during sample addition | Try to avoid cross-contamination when adding samples |
| The color of plate is chaotic and irregular | Cross-contamination from manual plate washing | When washing the plates by hand, the first 3 injections of the lotion should be discarded immediately, and the soaking time should be set for the next few times to reduce cross-contamination. |
| | Cross-contamination when clapping | Use a suitable absorbent paper towel when clapping the plate, do not pat irrelevant substances into the well of the plate, and try not to pat in the same position to avoid cross-contamination. |

| Description of results | Possible reason | Recommendations and precautions |
|---|---|---|

| | | |
|---|---|---|
| The color of plate is chaotic and irregular | The liquid filling head of the plate washer is blocked, resulting in unsatisfactory liquid addition or large residual amount of liquid suction, resulting in the color of plate is chaotic and irregular | Unblock the liquid addition head, so that each well is filled with washing liquid when washing the plate and the residual amount should be small when aspirating liquid. |
| | Incomplete centrifugation of the sample, resulting in coagulation in the reaction well or interference of sediment or residual cellular components | Serum plasma should be fully centrifuged at 3000 rpm for more than 6 minutes |
| | The sample is stored for too long time, resulting in contamination. | Samples should be kept fresh or stored at low temperature to prevent contamination |
| | Incorrect preparation of Washing Solution or direct misuse of concentrated Washing Solution | Please configure according to the manual |

## Declaration

1. Limited by current conditions and scientific technology, we can't conduct comprehensive identification and analysis of all the raw material provided. There might be some qualitative and technical risks for users using the kit.

2. This assay is designed to eliminate interference by factors present in biological samples. Until all factors have been tested in the ELISA immunoassay, the possibility of interference cannot be excluded.

3. The final experimental results will be closely related to the validity of products, operational skills of the operators, the experimental environments and so on. We are only responsible for the kit itself.

4. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions.

5. Incorrect results may occur because of incorrect operations during the reagents preparation and loading, as well as incorrect parameter settings of the Micro plate reader. Please read the instructions carefully and adjust the instrument prior to the experiment.

6. Even the same operator might get different results in two separate experiments. To get reproducible results, the operation of every step in the assay should be controlled.

7. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some variables such as transportation conditions, different lab equipment, and so on. Intra assay variance among kits from different batches might arise from the above reasons too.

8. Kits from different manufacturers or other methods for testing the same analyte could bring out inconsistent results, since we haven't compared our products with those from other manufacturers.

9. The kit is designed for research use only; we will not be responsible for any issues if the kit is applied in clinical diagnosis or any other related procedures.