DIASTAT® Anti-Thyroid Peroxidase (TPO)

For research use only. Not for use in diagnostic procedures.

distributed in the US/Canada by:
EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com • info@eaglebio.com

Document No. E-23-0121-07
January, 2014
ENGLISH: INTENDED USE

The DIASTAT® Anti-Thyroid Peroxidase (anti-TPO) test is a quantitative/qualitative enzymelinked immunosorbent assay (ELISA) for the detection of the IgG class of autoantibodies specific for thyroid peroxidase in human serum or EDTA, lithium heparin, and sodium citrate plasma. It is intended to aid in the diagnosis of autoimmune thyroid disorders and is not definitive in isolation. Autoantibody levels represent one parameter in a multicriterion diagnostic process.

INTRODUCTION

Autoimmune thyroid disorders encompass autoimmune destruction and stimulation; both states are associated with predominantly IgG local and circulating thyroid autoantibodies.

The presence of anti-thyroglobulin (Tg) autoantibodies in patients with Hashimoto’s thyroiditis was first demonstrated in 1956 by Roitt et al1 using gel diffusion precipitation. Trotter et al 2 and Roitt et al 3 subsequently showed that many patients with advanced thyroiditis had antibodies for a thyroid antigen distinct from Tg. This was termed thyroid microsomal antigen (TMA). Considerable evidence indicates that TMA is antigenically related to thyroid peroxidase (TPO), a membrane-bound glycoprotein enzyme with an approximate mass of 101kD, whose in vivo function is the iodination of tyrosine in the synthesis of the thyroid hormones T3 and T4. TMA and TPO may be identical moieties4-8; cloning of human TPO gives further support to their close identity9. TPO autoantibodies may play a pathogenic role in destructive autoimmune thyroid diseases as they can fix complement and consequently induce cytolysis10,11. Autoimmune reactivity to TPO is believed to be polyclonal, with autoantibodies recognising a minimum of six distinct determinants12. Anti-TPO antibodies are found, often in conjunction with anti-thyroglobulin autoantibodies, in the majority of Hashimoto’s Thyroiditis, Graves’ disease and in cases of Primary Myxoedema. The relationship of autoimmune thyroid disease in pregnancy has been the subject of considerable interest, with the demonstration of TPO antibodies in most cases of post-partum thyroiditis13-16 and the association of thyroid autoantibodies with increased miscarriage risk17. Anti-TPO antibodies are found in other non-thyroid conditions, e.g. pernicious anaemia 18,19, diabetes mellitus 20,21, rheumatoid arthritis 22, Addison’s disease 21 and Sjogren’s Syndrome 19. In addition, anti-TPO antibodies are detectable at low levels in 2-8% of apparently healthy individuals, particularly in the elderly and more often in women than in men, although the clinical significance of this is unclear.

PRINCIPLE OF THE ASSAY

The wells of the microtitre strips are coated with recombinant human TPO (rTPO) During the first incubation, specific autoantibodies in diluted serum or plasma bind to the antigen-coated surface. The wells are then washed to remove unbound components. In the second incubation, the Conjugate, enzyme-labelled antibodies to human IgG, binds any surface-bound autoantibodies. After further washing, specific autoantibodies are traced by incubation with the Substrate. Addition of Stop Solution terminates the reaction, resulting in a coloured end-product. The amount of Conjugate bound is measured in absorbance units. In the qualitative protocol, the amount of Conjugate bound by the sample is compared with that bound by the Reference Control. In the quantitative protocol, the concentration of anti-TPO autoantibody can be estimated by interpolation from a dose-response curve based on Standards. The Standards are calibrated against NIBSC 66/387 thyroid microsomal antibody reference preparation.
**KIT COMPONENTS**

<table>
<thead>
<tr>
<th></th>
<th>Component</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>IgG Conjugate</td>
<td>1 x 15 mL, Alkaline phosphatase-labelled antibodies to human IgG, Tris buffer, protein stabiliser, &lt;0.1% (w/v) sodium azide. Ready-to-use.</td>
</tr>
<tr>
<td>B</td>
<td>Substrate</td>
<td>1 x 15 mL, Mg²⁺, phenolphthalein monophosphate (PMP), buffer solution. Ready-to-use. Do not expose to light during storage.</td>
</tr>
<tr>
<td>C</td>
<td>Stop Solution</td>
<td>1 x 15 mL, Sodium hydroxide, EDTA, carbonate buffer (pH &gt;10). Ready-to-use.</td>
</tr>
<tr>
<td>D</td>
<td>Wash Buffer Concentrate (16X)</td>
<td>3 x 25 mL, Borate buffer, 0.4% (w/v) sodium azide. Dilute before use.</td>
</tr>
<tr>
<td>E</td>
<td>TPO-Coated Wells and Strip Holder</td>
<td>12 x 8 well microtitre strips, Coated with human recombinant TPO antigen, in a resealable foil pack with desiccant. Colour-coded RED. Individual wells can be broken off from each microtitre strip.</td>
</tr>
<tr>
<td>F</td>
<td>Sample Diluent Concentrate (5X)</td>
<td>1 x 25 mL, Phosphate buffer, protein stabiliser, 0.5% (w/v) sodium azide. Dilute before use.</td>
</tr>
<tr>
<td>1-5</td>
<td>Anti-TPO Standards</td>
<td>5 x 1.0 mL, Human plasma, buffer, &lt;0.1% (w/v) sodium azide. 0, 8, 25, 125, 500 IU/mL. Ready-to-use.</td>
</tr>
<tr>
<td>6</td>
<td>Anti-TPO Reference Control</td>
<td>1 x 1.5 mL, Human plasma, buffer, &lt;0.1% (w/v) sodium azide.</td>
</tr>
<tr>
<td>+/-</td>
<td>Positive Control</td>
<td>1 x 0.2 mL, Negative Control</td>
</tr>
</tbody>
</table>

**STORAGE OF REAGENTS**

*Opened Kit Stability*

A kit was opened and reused on three occasions over a three month period with no adverse effect on kit performance.

*Handling and Procedural Notes*

1. Store kit components at 2-8°C and use until the expiry date on the labels. Do not use expired reagents.
2. Do not mix different lot numbers.
3. Do not freeze kits.
4. Wash Buffer Concentrate, Sample Diluent Concentrate and Positive and Negative Controls must be diluted before use. All other reagents are ready-to-use.
5. Diluted Wash Buffer and diluted Sample Diluent are stable at 2-8°C for up to 6 months if microbial contamination is avoided.
6. Replace surplus microtitre strips in the foil pack and store with the desiccant at 2-8°C, until required.
7. The plate holder is adapted for use with snappable wells only.
8. Do not expose Substrate to light during storage.
9. Avoid contamination of reagents. Use a new disposable pipette tip for each reagent or sample manipulation.
**Indications of Deterioration**
The Substrate should be pale yellow in colour. Pink colouring indicates contamination and the reagent must be discarded. Turbidity or precipitation in any component indicates deterioration and the component should be discarded.

**Sample Collection and Storage**
The assay is recommended for serum or EDTA, lithium heparin and sodium citrate plasma samples; do not use lipaemic, haemolysed or turbid samples. Thoroughly mix thawed samples before assay and avoid repeated freeze/thawing. Do not heat-inactivate samples, this may yield false positive results.

Samples may be stored undiluted or at 1:101 dilution in diluted Sample Diluent at -20° C or 2-8° C for two weeks.

**WARNINGS AND PRECAUTIONS**

For *in vitro* diagnostic use only.

**Safety Precautions**

1. Adhere strictly to the instructions in this booklet, particularly for handling and storage conditions.
2. Standards and Controls contain human plasma tested by FDA-cleared assays for hepatitis B surface antigen, HCV, HIV antigen and HIV antibodies and found to be non-reactive/negative. As no known test offers complete assurance that infectious agents are absent, Standards and Controls should be considered potentially infectious and handled with the same precautions as any other potentially biohazardous material. The CDC/NIH Health Manual "Biosafety in Microbiological and Biomedical Laboratories", 3rd edition, 1993, describes how these materials should be handled in accordance with Good Laboratory Practice. This is applicable in the USA.
3. Do not pipette by mouth.
4. Do not smoke, eat, drink or apply cosmetics in areas where kits and samples are handled.
5. Any skin complaints, cuts, abrasions and other skin lesions should be suitably protected.
6. The Controls, Conjugate, Sample Diluent Concentrate and Wash Buffer Concentrate contain sodium azide which can react with lead and copper plumbing to form highly explosive metal azides. On disposal, drain with large quantities of water to prevent azide build-up.
7. The Stop Solution contains sodium hydroxide. Avoid contact with skin, eyes and mucous membranes. Spillage should be mopped up with copious amounts of water. If contact with skin or eyes occurs, irrigate with water and seek medical attention immediately.
8. The substrate contains PMP, Bronidox L and Diethanolamine. Avoid contact with skin, eyes and respiratory system. If contact with skin, eyes or respiratory system occurs, rinse with water and seek medical advice.
9. Material safety data sheets for all hazardous components contained in this kit are available on request from Euro Diagnostica.

**Warning**
Contains: Diethanolamine

- **H319:** Causes serious eye irritation.
- **P264:** Wash hands thoroughly after handling.
- **P280:** Wear protective gloves/protective clothing/eye protection/face protection.
- **P305+P351+P338:** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- **P337+P313:** If eye irritation persists: Get medical advice/attention.
C.

**Warning**
Contains: Sodium hydroxide

H315: Causes skin irritation.
H319: Causes serious eye irritation.
P264: Wash hands thoroughly after handling.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352: IF ON SKIN: Wash with plenty of soap and water.
P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P332+P313: If skin irritation occurs: Get medical advice/attention.
P337+P313: If eye irritation persists: Get medical advice/attention.

D. and F.

**Warning**
Contains: Sodium azide

H302: Harmful if swallowed.
EUH032: Contact with acids liberates very toxic gas.
H412: Harmful to aquatic life with long lasting effects.
P264: Wash hands thoroughly after handling.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P273: Avoid release to the environment.

**PREPARATION**

**Materials/Equipment Required but not Provided**

1. 96 well plate/strip reader with 550 nm filter (540-565 nm is acceptable).
2. Precision pipettes to dispense 10µL, 100µL, 1 mL. Automatic pipette to dispense 100µL. Automatic pipette to dispense 200µL for manual washing, automatic plate washer optional.
3. Glass/plastic measuring cylinders: 1×100 mL, 1×400 mL.
4. 1mL volume containers.
5. Distilled/deionised water.
7. Timer for 30 and 60 minute intervals.

**Preparation for the Assay**

Allow all kit components, including the microtitre strips, to warm up to 18-25°C for 30-60 minutes before use. Mix reagents by gentle inversion.

**Do not dilute the Reference Controls.**
Dilute the following reagents and mix thoroughly.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
<th>Add</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wash Buffer Concentrate</td>
<td>1 vial</td>
<td>375 mL distilled/deionised water</td>
</tr>
<tr>
<td>Sample Diluent Concentrate</td>
<td>1 vial</td>
<td>100 mL distilled/deionised water</td>
</tr>
<tr>
<td>Positive and Negative Controls/samples</td>
<td>10µL</td>
<td>1 mL diluted Sample Diluent</td>
</tr>
</tbody>
</table>

Microtitre wells are supplied in strips of eight. If other than a multiple of eight wells are required, proceed as follows.
1. Remove strip from holder by pushing underside of wells.
2. Snap off required number of wells.
3. Hinge rectangular hole into bottom edge (to H) of the holder groove.
4. Ensure the square hole, with nick on left, is firmly held along the top edge (row A).

ASSAY PROTOCOL

Qualitative protocol: run Reference Control, Positive and Negative Controls, and samples.
Quantitative protocol: run Standards (1-5), Positive and Negative Controls, and samples.

1. Reference wells for identification.
2. Pipette 100µL Reference Control/Standards in duplicate, pre-diluted Positive and Negative Controls, and pre-diluted patient samples into appropriate wells. Remember to change pipette tips between additions. This step should not exceed 15 minutes for any one set of Standards/Controls/samples.
3. Incubate 60±10 minutes at 18-25° C.
4. Decant strip contents by quick inversion over a sink suitable for the disposal of biological materials, bearing in mind the potential infective hazard of the samples. Blot inverted strips well with paper towels.
5. Wash wells five times with a minimum of 200µL diluted Wash Buffer. Decant and blot after each wash step.
6. Add 100µL IgG Conjugate 1 to each well.
7. Incubate 30±5 minutes at 18-25° C.
8. Repeat steps 4 and 5.
9. Add 100µL Substrate to each well.
10. Incubate 30±5 minutes at 18-25° C. Do not decant.
11. Add 100µL Stop Solution to each well, in the same order and rate as the Substrate. Tap wells gently to mix.
12. Read strips within 24 hours at 550nm (540-565nm).
CALCULATION AND INTERPRETATION OF RESULTS

Consider each assay separately when calculating and interpreting results. Calculate the absorbance value (optical density) ratio for the Positive and Negative Controls, and for each sample.

Absorbance Ratio = \frac{\text{Sample or Control Absorbance Value}}{\text{mean Reference Control Absorbance Value}}

Users should calculate a cut-off between positive and negative samples that is specific to their patient populations. Results from the patient populations used in the Euro Diagnostica clinical trial suggest the following cut-off:

<table>
<thead>
<tr>
<th>Absorbance Ratio</th>
<th>Result Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1.0</td>
<td>Negative</td>
</tr>
<tr>
<td>≥1.0</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Quantitative Protocol

Plot the mean absorbance value of each Standard against log_{10} Standard concentration (see following table) on suitable graph paper. Concentrations of Controls and samples can then be read from the standard curve; a typical plot is shown below for reference purposes, it must not be used for interpreting results. 4-parameter logistic (4PL), 5-parameter logistic (5PL), log/logit lin/lin or spline curve fits are also satisfactory.

Samples with absorbances above Standard 5 (500 IU/mL) are outside the range of the assay, and should be reported as >500 IU/mL, diluted and re-assayed, correcting for this further dilution factor.

NB: As in any assay measuring antibodies, this assay determines the activity of the antibody present in the sample, rather than the concentration. Activity can be affected by a number of parameters, such as antibody avidity.

<table>
<thead>
<tr>
<th>Standard Number</th>
<th>Concentration IU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>125</td>
</tr>
<tr>
<td>5</td>
<td>500</td>
</tr>
</tbody>
</table>

Typical Standard Curve

![Graph showing typical standard curve](image-url)
QUALITY CONTROL

Ensure that adequate maintenance and calibration of the plate-reader is performed according to the manufacturer’s instructions, and that the correct wavelength is employed.

Users should ensure they are fully acquainted with the instructions for the assay, particularly the Warnings and Precautions section, and the Handling and Procedural Notes. Users should demonstrate that they can obtain performance specifications for precision and reportable range of test results comparable to those established by the manufacturer before reporting patient test results. It is recommended that the pre-diluted Positive and Negative Controls are run in duplicate in all assays to monitor the quality of the test procedure. Run the ready-to-use Reference Control in duplicate in all assays.

Assuming the precision specifications described by the manufacturer are met, failure of any Control to meet the Control ratio specifications below renders the assay invalid and patient results should not be reported. The operator may repeat the assay, having reviewed their procedure, or contact the distributor/manufacturer. If repeating the assay, prepare a fresh dilution of each Control and sample. Laboratories may wish to include in-house controls in each assay run. Store such control material at or below -20° C and avoid repeat freeze/thaw cycles. Preservatives such as sodium azide at 0.1% (w/v) will not affect sample results.

Levels of analytes identified in particular diseases are those established by the manufacturer for specific populations, and may not necessarily mirror the literature. Incidence levels, their relationship to specific diseases, reference ranges, and appropriate cut-off points should all be calculated for the specific populations serviced by users.

### Protocol Specifications

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qualitative (ratios)</td>
<td>Positive Control Absorbance = see Positive Control label</td>
</tr>
<tr>
<td></td>
<td>Reference Control Absorbance</td>
</tr>
<tr>
<td></td>
<td>Negative Control Absorbance</td>
</tr>
<tr>
<td></td>
<td>Reference Control Absorbance &lt;1.0</td>
</tr>
<tr>
<td>Quantitative</td>
<td>See Positive Control label for acceptable expected range (IU/mL)</td>
</tr>
<tr>
<td></td>
<td>Negative Control concentration &lt;10 IU/mL</td>
</tr>
</tbody>
</table>

EXPECTED VALUES

172 serum samples from asymptomatic apparently healthy donors, with an age range of 21 – 51 years, comprising approximately equal numbers of males and females, were tested for the presence of anti-TPO autoantibodies using the DIASTAT test and a further commercially available device. Sixteen (7%) samples were positive in the further test device and were omitted from the calculation of the DIASTAT reference range. Of the remaining 156 results, 153 (98%) gave values of less than 10 IU/mL. On the basis of this data the suggested cut-off for the DIASTAT anti-TPO ELISA is less than 10 IU/mL. The reference range is suggested as a guideline only, and each laboratory should establish a reference range appropriate to their patient populations and clinical practice. Concentrations are expressed in units derived from standards calibrated against the NIBSC 66/387 thyroid microsomal antibody reference preparation.

<table>
<thead>
<tr>
<th>Reference Range</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 IU/mL = Negative</td>
<td></td>
</tr>
<tr>
<td>≥10 IU/mL = Positive</td>
<td></td>
</tr>
</tbody>
</table>
**PERFORMANCE DATA**

**Concordance Study**

The performance of the DIASTAT Anti-TPO test was compared with a commercially available test for the measurement of autoantibodies to TPO. A total of 377 samples were evaluated, encompassing a spectrum of thyroid-associated diseases and those from an asymptomatic population. The following results were obtained:

<table>
<thead>
<tr>
<th>Immunoassay anti-TPO</th>
<th>DIASTAT ANTI-TPO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve</td>
</tr>
<tr>
<td>+ve</td>
<td>169</td>
</tr>
<tr>
<td>-ve</td>
<td>9</td>
</tr>
</tbody>
</table>

Co-positivity = 94.9%
Co-negativity = 95.5%
Overall agreement = 95.2%

**Clinical Sensitivity**

Clinical sensitivity was evaluated by testing 51 samples from patients diagnosed with Hashimoto's disease and 52 from patients with Graves' disease. Diagnosis was based on the laboratories’ diagnostic criteria.

49/51 (96.1%) of the patients with Hashimoto's disease tested positive using DIASTAT Anti-TPO.

38/52 (73%) of the patients with Graves' disease tested positive using DIASTAT Anti-TPO. The distribution of the anti-TPO results in the normal, Graves and Hashimoto populations is given below.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
<th>0-9.9 IU/mL</th>
<th>10-14.9 IU/mL</th>
<th>15-99.9 IU/mL</th>
<th>100-249.9 IU/mL</th>
<th>250-500 IU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals</td>
<td>153</td>
<td>153 (100%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Graves'</td>
<td>52</td>
<td>14 (27%)</td>
<td>6 (12%)</td>
<td>5 (10%)</td>
<td>8 (15%)</td>
<td>19 (37%)</td>
</tr>
<tr>
<td>Hashimoto's</td>
<td>51</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td>8 (16%)</td>
<td>14 (27%)</td>
<td>26 (51%)</td>
</tr>
</tbody>
</table>

**Dilution Characteristics**

Four dilutions of two patient samples were assayed using two kit batches. The following table shows the mean values obtained and the dilution-corrected recovery.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Mean Value IU/mL</th>
<th>Dilution Corrected % Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>344.6</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>A/2</td>
<td>163.2</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>A/4</td>
<td>80.5</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>A/8</td>
<td>41.7</td>
<td>97</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>449.8</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>A/2</td>
<td>221.7</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>A/4</td>
<td>102.7</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>A/8</td>
<td>51.3</td>
<td>91</td>
</tr>
</tbody>
</table>
Imprecision

1. **Intra-assay imprecision** determined by testing three controls in 20 assays, using one operator and two kit batches.

<table>
<thead>
<tr>
<th>Control</th>
<th>Mean Value IU/mL</th>
<th>RMS %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.8</td>
<td>5.9%</td>
</tr>
<tr>
<td>2</td>
<td>88.0</td>
<td>4.5%</td>
</tr>
<tr>
<td>3</td>
<td>243.3</td>
<td>4.4%</td>
</tr>
</tbody>
</table>

2. **Inter-assay imprecision** determined by testing three controls in 20 assays, using one operator and two kit batches.

<table>
<thead>
<tr>
<th>Control</th>
<th>Mean Value IU/mL</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.8</td>
<td>1.0</td>
<td>7.6%</td>
</tr>
<tr>
<td>2</td>
<td>88.0</td>
<td>7.9</td>
<td>9.0%</td>
</tr>
<tr>
<td>3</td>
<td>243.3</td>
<td>25.5</td>
<td>10.5%</td>
</tr>
</tbody>
</table>

Lower Limit of Detection
The lower limit of detection, calculated as the mean of the zero standard plus two standard deviations, run in replicates of 20 in two kit batches, was calculated as 1.0 IU/mL.

Interferences
Haemolysate up to 4.0 mg/mL, bilirubin up to 0.2 mg/mL, intralipid up to 15 mg/mL and the presence of rheumatoid factor up to 200 IU/mL produced results within ±10% of the target value.

Limitations of Use

1. Although the presence of antibodies to thyroid peroxidase is indicative of thyroid autoimmune disease, the data must be considered in light of other clinical and laboratory findings.
2. Some individuals may have high levels of anti-TPO antibodies with little or no evidence of clinical disease. By contrast, some patients with thyroid autoimmune disease may have undetectable levels of these antibodies.
3. Anti-TPO may be found in apparently healthy individuals. The clinical significance of this information is currently unclear.
4. For repeat patient sampling, e.g. for monitoring, the same type of sample (serum or EDTA, heparin or citrated plasma) should be used throughout the study period.
REFERENCES


SUMMARY OF PROTOCOL

1. Dilute samples and Positive and Negative Controls 1:101. Do not dilute Standards or Reference Control.
2. Add 100µL of Reference Control/Standards in duplicate, pre-diluted Positive and Negative Controls and samples into referenced wells of the microtitre strip.
3. Incubate 60±10 minutes at 18-25° C.
4. Wash strips 5 times.
5. Add 100µL of IgG Conjugate 1 to each well.
6. Incubate 30±5 minutes at 18-25° C.
7. Wash strips 5 times.
8. Add 100µL of Substrate to each well.
9. Incubate 30±5 minutes at 18-25° C.
10. Add 100µL of Stop Solution to each well.
11. Read absorbance at 550 nm
**FTPO 300, E-23-0121-07**

**CONJ**
Conjugate/Conjugado / Konjugat / Conjugato / Konjugat

**SUBS**
Substrate/Substrato / Substrat / Substrato / Substrat

**SOLN**
Stop solution/Solution d’Arrêt / Solución de Parada / Stoplösung/Soluzione bloccante / Stoplösning

**BUF**
Wash buffer concentrate (16 X)/Concentré tampon de (16X lavage)/Concentrado de Búfer de lavado (16X)/Washpuffer-Konzentrat (16 X)/Tampone di lavaggio concentrato (16 X)/Tvättbuffert koncentrat (16 X)

**Ag**
TPO-coated wells and strip holder/Cupules enduites de POT et Portebandes/Soporte para Bandas y Vasos/Recubiertos con TPO/TPO-beschichtete Vertiefungen und Streifenrahmen/Pozzetti rivestiti di TPO e supporto per strip/TPO-klädda brunnar och striphållare

**DIL**
Sample Diluent Concentrate (5 X)/Concentré diluent pour échantillons (5 X)/Concentrado de Diluente de Muestra (5 X)/Probendiluens Konzentrat/Diluente per campioni concentrato (5 X)/Provspädningsbuffert koncentrat (5 X)

**CAL**

**CONTROL**
Anti-TPO Reference Control/Témoin de référence anti-POT/Control de Referencia Anti-TPO/Anti-TPO Referenzkontrolle/Controllo di riferimento anti-TPO/Anti-TPO referenskontroll

**CONTROL**
Positive Controls/Témoins positifs / Controles Positivos / Positiv-Kontrollen / Controlli Positivi /Positiva kontroller

**EURO DIAGNOSTICA AB**
Lundavägen 151, SE-212 24 Malmö, Sweden
Phone: +46 40 53 76 00, Fax: +46 40 43 22 88
E-mail: info@eurodiagnostica.com
www.eurodiagnostica.com