**TRIAMCINOLONE ACETONIDE**

*5081TRIA[3]08.15*

A competitive enzyme immunoassay for screening and quantitative analysis of triamcinolone in urine

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## A competitive enzyme immunoassay for screening and quantitative analysis of triamcinolone in urine

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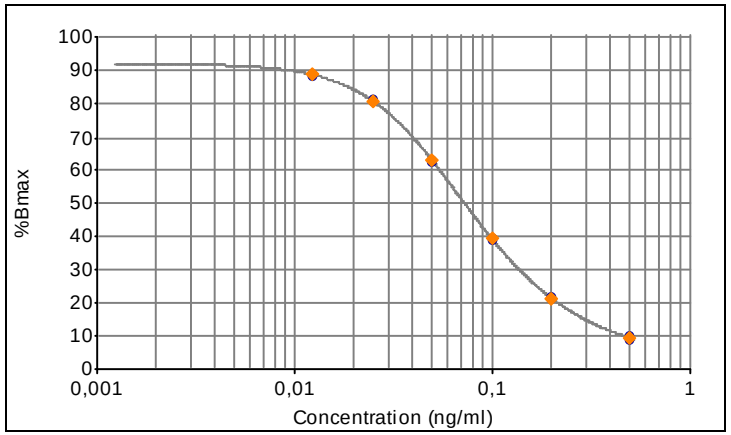


Figure 1 : Example of a calibration curve

8 Urine samples

The triamcinolone acetonide equivalents, as read from the standard curve, have to be multiplied by a factor 10 to obtain the triamcinolone acetonide content in urine samples.

## 12. ORDERING INFORMATION

For ordering the Triamcinolone Acetonide kit, please use cat. code 5081TRIA.

## 13. LAST MUTATIONS

Chapter: Equipment and materials required is added.
Chapter: Precautions is added.
Calibration curve.
Pipette schedule of the standard curve is adapted.
Updated lay-out.

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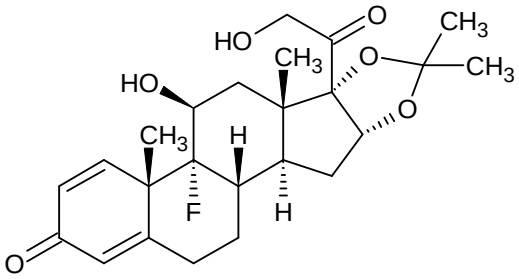
## BRIEF INFORMATION

The Triamcinolone ELISA is a competitive enzyme immunoassay for the screening of urine samples. The test is based on antibodies directed against triamcinolone.
The ELISA kit contains a 96 well microtiter plate as well as all essential reagents including ready-to-use standards to perform the test. Methods for a fast and efficient extraction of triamcinolone acetonide from different matrices are included in the kit manual.

## 1. INTRODUCTION




*Chemical structure of triamcinolone acetonide*

Triamcinolone acetonide is a synthetic glucocorticoid which is used as an anti-inflammatory drug. Corticosteroids such as dexamethasone and triamcinolone have growth and performance enhancing properties. Triamcinolone is banned from livestock farming as well as for doping purposes in sports.

## 2. PRINCIPLE OF THE TRIAMCINOLONE KIT

The kit is based on a microtiter plate (12 strips, each 8 wells), precoated with sheep antibodies to rabbit IgG.
In one incubation step, specific antibodies (triamcinolone), enzyme labelled triamcinolone (TRIA-HRP) and triamcinolone standards or sample are added to the precoated wells. The specific antibodies are bound by the immobilized rabbit antibodies and at the same time free triamcinolone (in the standard solution or in the sample) and enzyme labelled triamcinolone compete for the specific antibody binding sites (competitive enzyme immunoassay).
After an incubation of 30 minutes, the non-bound (enzyme labelled) reagents are removed in a washing step. The amount of bound enzym conjugate is visualized by the addition of a substrate/chromogen solution (tetramethylbenzidine/TMB). Bound enzyme transforms the chromogen into a coloured product.
The substrate reaction is stopped by the addition of sulfuric acid. The colour intensity is measured photometrically at 450 nm and is inversely proportional to the triamcinolone concentration in the sample.

## 3. SPECIFICITY AND SENSITIVITY

The triamcinolone ELISA utilizes a specific antibody raised in rabbit against protein conjugated triamcinolone acetonide. The reactivity pattern of the antibody is:

| Cross-reactivity: | |
|---|---|
| triamcinolone acetonide | 100% |
| fluocinolone acetonide | 26.7% |
| 6α-methylprednisolone | 0.02% |
| triamcinolone | 0.02% |
| cortisone | < 0.01% |
| dexamethasone | < 0.01% |
| prednisolone | < 0.01% |
| isoflupredone | < 0.01% |
| prednisolone | < 0.01% |
| betamethasone | < 0.01% |
| flumethasone | < 0.01% |
| fluoromethonolone | < 0.01% |
| beclomethasone | < 0.01% |
| beclomethasone dipropionate | < 0.01% |
| clobetasol propionate | < 0.01% |

The Limit of detection (LOD) is calculated as: Xn+3SD and is determined under optimal conditions.

| Matrix | Procedure | LOD (ppb) |
|---|---|---|
| Urine | 8 | 0.1 |

## 4. HANDLING AND STORAGE

- Kit and kit components are stored in a refrigerator (2°C to 8°C) before and immediately after use.
- After the expiry date of the kit and/or components has passed, no further quality guarantee is valid.
- Bring all kit components including the microtiter plate at ambient (room) temperature before use.
- Avoid condensation in the wells of the plate. Bring the sealed plate at ambient temperature before opening the plate sealing.
- Any direct action of light on the chromogen solution should be avoided.

Degeneration of the reagents may have occurred when the following phenomena are observed:

- A blue colouring of the chromogen solution before transferring it into the wells.
- A weak or absent colour reaction of the maximum binding (zero standard) (E450nm < 0.8).



6. Seal the microtiter plate and shake the plate for 1 minute on a microtiter plate shaker.

7. Incubate the place for 30 minutes at 20ºC to 25ºC.

8. Discard the solution from the microtiter plate and wash 3 times with rinsing buffer.

9. Pipette 100 µl of substrate solution to each well.

10. Incubate for 15 minutes in the dark at 20ºC to 25ºC.

11. Pipette 100 µl of the stop solution to each well.

12. Read the absorbance values immediately at 450 nm.

## 11. INTERPRETATION OF RESULTS

The O.D. values of the six standards and the samples (mean values of the duplicates) are divided by the mean O.D. value of the zero standard (Bmax, wells A1 and A2) and multiplied by 100. The zero standard (Bmax) is thus made equal to 100% (maximal absorbance) and the other O.D. values are quoted in percentages of the maximal absorbance.

$$\frac{\text{O.D. standard (or sample)}}{\text{O.D. zero standard (Bmax)}} \times 100\% = \%\ \text{absorbance}$$

Calibration curve:
The values (% maximal absorbance) calculated for the standards are plotted on the Y-axis versus the analyte equivalent concentration (ng/ml) on a logarithmic X-axis.

Alternative for calibration curve:
The absorption value of the standards is plotted on the Y-axis versus the concentration on the X-axis. The Y-axis is in logit the Y-axis is logarithmic.

## 10. ASSAY PROCEDURE

Rinsing protocol

In ELISA's, between each immunological incubation step, unbound components have to be removed efficiently. This is reached by appropriate rinsing. It should be clear that each rinsing procedure must be carried out with care to guarantee good inter- and intra-assay results.
Basically, manual rinsing or rinsing with automatic plate wash equipment can be performed as follows:

Manual rinsing

1. Empty the contents of each well by turning the microtiter plate upside down and remove residual liquid by striking the plate against a paper towel.
2. Fill all the wells to the rims (300 µl) with rinsing solution.
3. This rinsing cycle (1 and 2) should be carried out 3 times.
4. Turn the plate upside down and empty the wells by a firm short vertical movement.
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove residual rinsing solution from the wells.
6. Take care that none of the wells dry out before the next reagent is dispensed.

Rinsing with automatic microtiter plate wash equipment

When using automatic plate wash equipment, check that all wells can be aspirated completely, that the rinsing solution is nicely dispensed reaching the rim of each well during each rinsing cycle. The washer should be programmed to execute three rinsing cycles.

Assay protocol

1. Prepare samples according to Chapter 8 (Sample preparation) and prepare reagents according to Chapter 9 (Preparation of reagents).

2. Pipette 100 µl of zero standard in duplicate (wells H1, H2, blank).
   Pipette 50 µl of zero standard (Bmax) in duplicate (wells A1, A2).
   Pipette 50 µl of each of the standard solutions in duplicate (wells B1,2 to G1,2 i.e. 0.0125, 0.025, 0.05, 0.1, 0 and 0.5 and ng/ml).

3. Pipette 50 µl of each sample solution in duplicate into the remaining wells of the microtiter plate .

4. Pipette 25 µl of enzyme conjugate (TRIA-HRP) to all wells, except H1 and H2.

5. Add 25 µl triamcinolone acetonide antibody to all wells except wells H1 and H2.

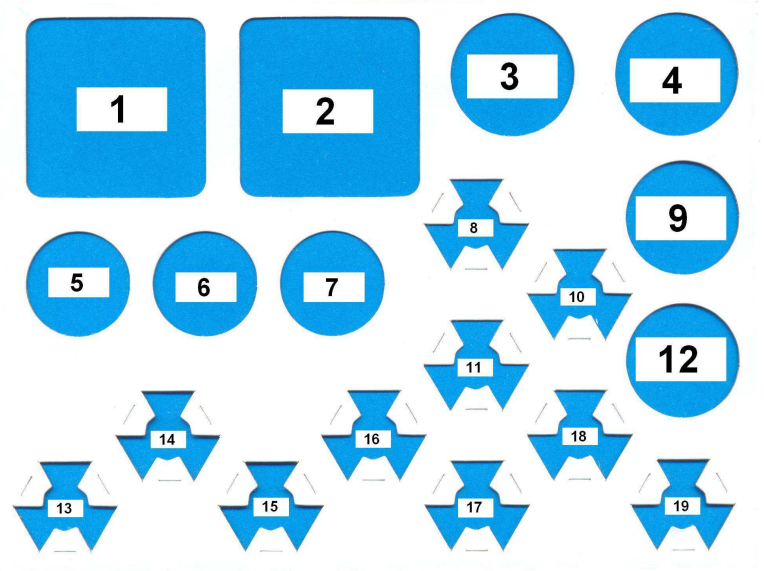


## 5. KIT CONTENTS

Manual

One sealed microtiter plate (12 strips, 8 wells each), coated with antibodies to rabbit IgG. Plate is ready-to-use.

Position of the reagents in the kit. For preparation of the reagents see Chapter 9.




1. **Dilution buffer** (30 ml, 10x concentrated)
2. **Rinsing buffer** (30 ml, 20x concentrated
3. **Substrate solution** (12 ml, ready-to-use)
4. **Stop solution** (15 ml, ready-to-use)
5. **Antibody** (lyophilized, yellow cap)
6. not in use
7. not in use
8. **Conjugate** (0.1 ml, 100x concentrated)
9. not in use
10. not in use
11. not in use
12. not in use
13. **Zero standard** (2 ml ready-to-use)
14. **Standard solution 1** (1 ml ready-to-use) **0.0125 ng/ml**
15. **Standard solution 2** (1 ml ready-to-use) **0.025 ng/ml**
16. **Standard solution 3** (1 ml ready-to-use) **0.05 ng/ml**
17. **Standard solution 4** (1 ml ready-to-use) **0.1 ng/ml**
18. **Standard solution 5** (1 ml ready-to-use) **0.2 ng/ml**
19. **Standard solution 6** (1 ml ready-to-use) **0.5 ng/ml**

## 6. EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- Scales and weighing vessels
- Gloves
- Fume hood
- Homogeniser (vortex, mixer)
- Centrifuge (2000 x g)
- Automated microtiter plate washer or 8-channel micropipette 100 – 300 µl
- Microtiter plate shaker
- Microtiter plate reader with 450 nm filter
- Micropipettes, 100 – 1000 µl
- Multipipette with 2.5 ml combitips

## 7. PRECAUTIONS

- Triamcinolone is a toxic compound. Avoid contact with mouth and skin.
- The stop reagent contains 0.5 M sulfuric acid. Do not allow the reagent to get into contact with the skin.
- Avoid contact of all biological materials with skin and mucous membranes.
- Do not pipette by mouth.
- Do not eat, drink, smoke, store or prepare foods, or apply cosmetics within the designated work area.
- TMB is toxic by inhalation, in contact with skin and if swallowed; observe care when handling the substrate.
- Do not use components past expiration date and do not intermix components from different serial lots.
- Each well is ultimately used as an optical cuvette. Therefore, do not touch the under surface of the wells, prevent damage and dirt.
- All components should be completely dissolved before use. Take special attention to the substrate, which crystallises at 4°C.
- Optimal results will be obtained by strict adherence to this protocol. Careful pipetting and washing throughout this procedure are necessary to maintain good precision and accuracy.



## 8. SAMPLE PREPARATION

**Alternative methods may be used.**

Urine samples

Urine samples can be applied directly after a 10 times dilution in dilution buffer.

- All samples are centrifuged for 5 minutes at 2000 x g
- Pipette 50 µl of the supernatant into a glass tube, add 450 µl of dilution buffer and mix thoroughly (10 times diluted samples)
- Use 50 µl of diluted sample per wells in the ELISA.

## 9. PREPARATION OF REAGENTS

Before starting the test, allow the reagents to come to ambient temperature. Any reagents not used should be put back into storage immediately at 2°C to 8°C.

Microtiter plate

Return unused strips into the resealable bag with desiccant and store at 2°C to 8°C for use in subsequent assays. Retain also the strip holder.

Dilution buffer

The dilution buffer is delivered 10 times concentrated (e.g. 1ml of concentrated buffer + 9 ml of distilled water). Prepare dilutions freshly before use.

Antibody solution

Reconstitute the vial of lyophilized antibodies with 4 ml dilution buffer, mix thoroughly and store the vial immediately after use in the dark at 2°C to 8°C. If the antibody component is to be stored for more than one week, this component must be stored in aliquots at –20°C immediately after the first use.

Conjugate solution

The conjugate is delivered 100x concentrated. Spin down the conjugate in the vial by a short centrifugation step (1 minute, 1000 x g). Add 5 µl of the concentrated conjugate solution to 495 µl dilution buffer. Per 2 x 8 wells 400 µl is required. Store unused concentrated conjugate at 2°C to 8°C.

Rinsing buffer

The rinsing buffer is delivered 20 times concentrated. Prepare dilutions freshly before use. Per strip 40 ml of diluted rinsing buffer is used (2 ml concentrated rinsing buffer + 38 ml distilled water).

Substrate

The substrate solution is ready-to-use but precipitates at 4°C. Make sure that this vial is at room temperature (keep in the dark) and mix the content before pipetting into the wells.