Application Note:
AN-MR-MSFC12-1210

Immunochemical determination of 17ß-estradiol from bovine urine samples using Thermo Scientific microplate instruments

Key Words:
- 17ß-estradiol
- ELISA
- Food safety

Abstract
This note describes how to perform a 17ß-estradiol screening assay from bovine plasma using Thermo Scientific microplate instrumentation.

Introduction
17ß-estradiol is a natural, low-level sexual hormone in bovine blood, at the level of a few pg/ml, except in pregnant animals. Therefore, a high hormone level in a non-pregnant animal can indicate that it has been illegally treated with an anabolic agent to increase its growth rate.
The European Council limits the use of 17ß-estradiol for medical treatment of slaughtered animals because it has been found to be a potential carcinogen[1]. Therefore, the Finnish Food Safety Authority tests for this hormone in the animals to be slaughtered.

RIDASCREEN® 17ß-Estradiol is a competitive enzyme immuno-assay for the quantitative analysis of 17ß-estradiol in bovine plasma. The basis of the test is an antigen-antibody reaction and absorption is inversely proportional to the 17ß-estradiol concentration in the sample. The illustration describing the principle of the assay is shown in Figure 1.

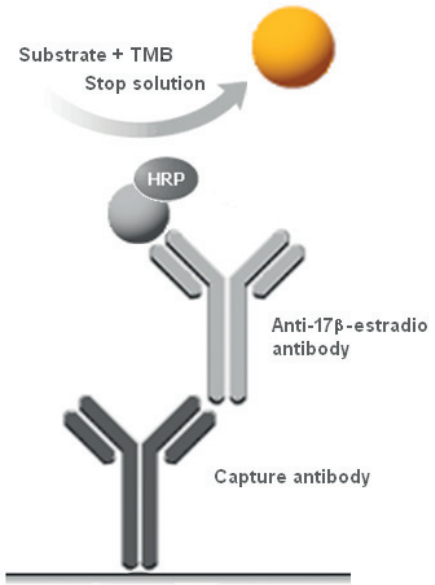


Figure 1. Estradiol assay principle

Materials and methods
- RIDASCREEN® 17ß-Estradiol R-Biopharm AG (Cat. code R 2301)
- Thermo Scientific Multiskan FC microplate photometer (Thermo Scientific code 51119000)
- Thermo Scientific Wellwash microplate washer (Thermo Scientific code 5165000)
- Thermo Scientific Multidrop Combi reagent dispenser (Thermo Scientific code 5840300)
- Bovine plasma samples
- Thermo Scientific F1 Manual Pipette (Thermo Scientific code 4641080)

The assay was performed at the Chemistry and Toxicology Unit of the Finnish Food Safety Authority at Viikki, Helsinki (EVIRA) as part of the normal screening procedure of bovine samples.

The kit measurement range covers 0.05–12.8 µg/l. The cut-off value used at EVIRA is 0.1 µg/l

The sample treatment was made according to the kit instructions: 1 ml of plasma was extracted in 5 ml tert-butylmethyl ether-petroleum ether 30 + 70 (v/v). The phase was evaporated and the residue dissolved in 400 µl of buffer. 20 µl was employed for the test. Several controls, spiked to a concentration of 0.2 ng/ml, are always included in the assay.

Because of the sample preparation, a dilution factor of 0.4 was used for the bovine plasma samples.

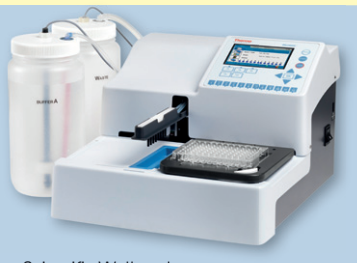
The basic assay procedure and the instrumentation used are described in Table 1.

Table 1. Assay procedure and instrumentation

1. The Thermo Scientific microtiter wells are coated with a secondary antibody against anti-17ß-estradiol antibodies. The standards and samples,17ß-estradiol enzyme conjugate and anti-17-ßestradiol antibodies, are added in the first assay phase. Incubation is performed for 2 h at RT.

Thermo Scientific F1 Pipette and/or Multidop Combi

2. In the second phase, the unbound enzyme conjugate is removed by washing.



Thermo Scientific Wellwash

3. The enzyme substrate (urea peroxide) and chromogen (tetramethylbenzidine) are added to the wells. Incubation for 30 min at RT. Bound enzyme conjugate converts the chromogen into a blue product.

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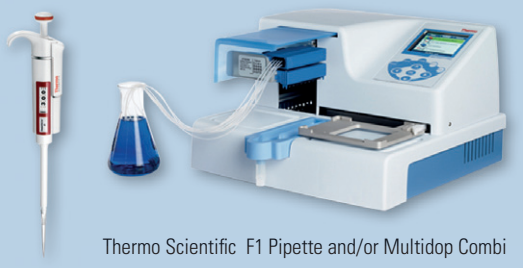
4. The addition of the stop solution leads to a color change from blue to yellow.



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5. Finally, the absorbance measurement is performed at 450 nm.

Thermo Scientific Multiskan FC

When performing this type of screening, the most important objective is to secure all positive samples and avoid false negative results. Therefore, even though this kit has been found to have a very high recovery percentage (142–504%[2]), it can be considered suitable for its intended use.

At EVIRA, all positive samples are confirmed with GC-MS.

Results

The calculations were performed according to the kit instructions supplied with Thermo Scientific SkanIt Software as described in Figure 2.

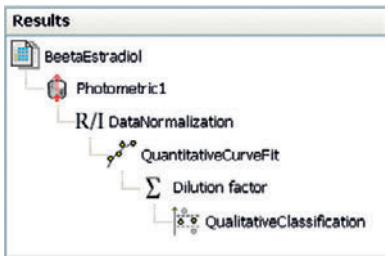

Figure 2. SkanIt Software calculations

1. Data normalization

The zero standard is set equal to 100% and the absorbance values are given in percentages.

2. Quantitative curve fit

The calibration curve was fitted using the four-parameter logistics-fitting formula.
An example of a calibration curve is shown in Figure 3.

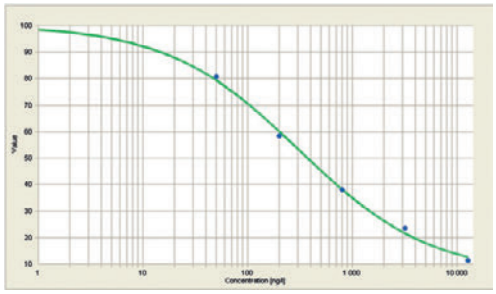

Figure 3. An example of a RIDASCREEN® 17ß-Estradiol calibration curve

3. User equation

With a user-defined equation, it is possible to create an unlimited number of variables that can be used in the formula. In this assay, the dilution factor 0.4 was added with a user equation.

4. Qualitative classification

The samples can be categorized as either positive or negative, according to their relation to the cut-off limit, using the qualitative classification step. With this step, the positive samples can easily be identified from the results.

As a final result, all of the spike controls obtained a positive status with a concentration range of: 160–430 ng/l. In addition to those, 10 unknown samples were categorized as positive. Those samples were sent to be re-tested by GC-MS.

The assay protocol for the 17ß-estradiol can be downloaded from the Multiskan FC protocol library: http://www.thermoscientific.com/skanit.

References

1. European Union (1996). – Council Directive 96/22/ EC of 29 April 1996 concerning the prohibition on the use in stock farming of certain substances having a hormonal orthyrostatic action and of ß-agonists, and repealing Directives 81/602/ EEC, 88/146/EEC and 88/299/EEC. Off. J. Eur. Union, L 125 of 23.5.1996, 3–9.

2. Simontacchi, C et al. Accuracy in naturally occurring anabolic steroid assays in cattle and first approach to quality control in Italy Analyst, 1999, 124, 307–312.

Further information

For further information on the Thermo Scientific Multiskan FC microplate photometer, the Wellwash microplate washer, the Multidrop Combi reagent dispenser and Thermo Scientific Finnpipettes, visit www.thermoscientific.com.

For further information about the reagents, refer to www.r-biopharm.com.

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