Progesterone

ELISA kit

(96 Tests)

ZELLX GmbH (Germany)

CAT No. ZX-55103-96

www.zellx.de

Sample Types Validated for:

Urine, Dried Fecal Extracts, and Tissue Culture Media

!!! Caution: This product is for Research Use Only. Not for *in vitro* Diagnostics !!!

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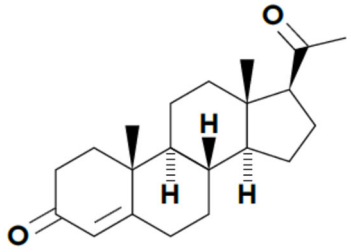
Please read this insert completely prior to using the product.

Introduction

Background

Progesterone ($C_{21}H_{30}O_2$) is a C-21 steroid hormone which belongs to a class of hormones called progestogen. Progesterone is the major naturally occurring human progestogen, and an essential regulator of human female reproductive function in the uterus, ovary, mammary gland and brain, involving in the female menstrual cycle, gestation and embryogenesis. Progesterone also plays an important role in non-reproductive tissues such as the cardiovascular system, bone and the central nervous system. For instance, to perform its neurotrophic effects in the peripheral nervous system, Progesterone activates the growth and maturation of axons and stimulates the repair and replacement of myelin sheaths in regenerating nerve fibers. Progesterone action is conveyed by two isoforms of the nuclear Progesterone receptor (PR), PRA and PRB. PRA and B are expressed in a variety of normal breast tissue from humans, rats and mice.



Assay principle

The ZellX® Progesterone Immunoassay kit is designed to quantitatively measure Progesterone present in urine, extracted dried fecal samples, and tissue culture media. A Progesterone stock solution is provided to generate a standard curve for the assay and all samples should be read off the standard curve.

The kit includes a 96-well plate that is pre-coated with a secondary anti-mouse antibody. The function of this antibody is to capture the mouse anti-Progesterone antibody bound to Progesterone conjugate (peroxidase-labeled) and hold this complex to the plate during the subsequent detection steps. The Progesterone-conjugate (labeled) and the sample Progesterone (unlabeled) compete for binding to the mouse antibody. After 2 hours of incubation, the substrate is added to react with the peroxidase-labeled antibody-antigen conjugate. After stopping the reaction, the intensity of the generated color can be measured at 450 nm. The lower the amount of Progesterone in the sample, the stronger the signal due to more labeled Progesterone bound to the well.

General information

Materials supplied in the Kit

Component	*Quantity*
Progesterone Standard (32 ng/mL)	125 µL
Progesterone Antibody	2.6 mL
Progesterone Conjugate	2.6 mL
Assay Buffer Concentrate (5x)	11 mL
Wash Buffer Concentrate (20x)	25 mL
TMB Substrate	11 mL
Stop Solution	5 mL
Coated Clear 96-Well Plate & Sealer	1 plate

Storage instruction

All reagents should be stored at 4° C until the expiration date of the kit.

Materials required but not supplied

Deionized water (diH_2O)

Phosphate Buffer Saline (PBS)

Microplate/ELISA reader capable of reading optical absorption at 450 nm

Microplate shaker, Centrifuge, Vortex mixer

Precision pipettes, multichannel/repeater pipettes and disposable pipette tips

For Dried Fecal Sample:

ACS Grade Ethanol

Glass test tubes

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

Stop Solution is an acidic solution and should not come in contact with skin or eyes. Handling this reagent needs appropriate precaution.

General remarks

- Equilibrate all kit components to room temperature (RT) 30 minutes before use.
- The instruction must be strictly followed.
- The reading of Microplate/ELISA reader must be set at the appropriate wavelength.
- Pipette tips should not be used more than once in order to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.
- The antibody-coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.
- This kit utilizes a peroxidase-based readout system. Buffers, including Wash Buffers from other manufacturers, containing sodium azide will inhibit color production by the enzyme. Make sure all buffers used for samples are azide-free. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer.

Assay protocol

Reagent preparation

i. **Assay Buffer:** Prepare a 1:5 dilution of Assay Buffer Concentrate with diH_2O (1 part Assay Buffer Conc. with 4 parts diH_2O), and mix well. Assay Buffer can be stored at 4°C for up to 3 months.

ii. **Wash Buffer:** Prepare a 1:20 dilution of Wash Buffer Concentrate with diH_2O (1 part Wash Buffer Conc. with 19 parts diH_2O), and mix well. Assay Buffer can be stored at room temperature for up to 3 months.

Sample preparation

This assay has been validated for dried fecal, urine and for tissue culture samples. Samples containing visible particulate should be centrifuged prior to using. Progesterone can be assayed in other sample types and preparation sample method please contact us at technical@zellx.de.

Since Progesterone is identical across all species, it is expected that this kit can measure Progesterone in human and other species.

Samples containing visible particulate should be centrifuged prior to conducting the assay.

All samples and standards must be used within 2 hours of preparation.

I. **Urine:**

- Urine should be diluted ≥ 1:4 by taking one part of sample and adding 3 or more parts of Assay Buffer prior to conducting assay.
- **Normalize the sample value based on creatinine levels using our Urine Creatinine assay kit Cat NO. ZX-44110-96 in random urine specimen.**

II. **Dried Fecal Sample:**

- Ensure that the sample is completely dry, and powder the sample to improve extraction recovery. Remove any large particles if possible.
- Weigh out ≥ 0.2 gram of dried fecal solid into a tube. Samples can be dried by passive drying, gentle heating (≤ 60 °C), or freeze-drying (lyophilization).
- Add 1 mL of ethanol per 0.1 gram of solid fecal sample (100 mg/mL) and seal.
- Shake strongly for at least 30 minutes.
- Centrifuge at 5000 rpm at 4°C for 15 minutes and collect supernatant in a clean tube. This material can be stored at ≤ -20°C for at least a month if properly sealed.
  - **Note:** Samples containing low levels of analyte can be concentrated by drying down the extract and resuspension in a reduced volume of Assay Buffer.
- Supernatant should be diluted ≥ 1:5 by taking one part of sample and adding 4 or more parts of Assay Buffer.
- Vortex well and allow to rest 5 minutes at room temperature
- Repeat the vortex step 2 more times to ensure complete steroid solubility.
- The final concentration of ethanol in the sample to be added to the wells should be ≤ 5%. **(≥ 1:4 dilution with Assay Buffer is needed.)**

III. **Tissue Culture Media:**

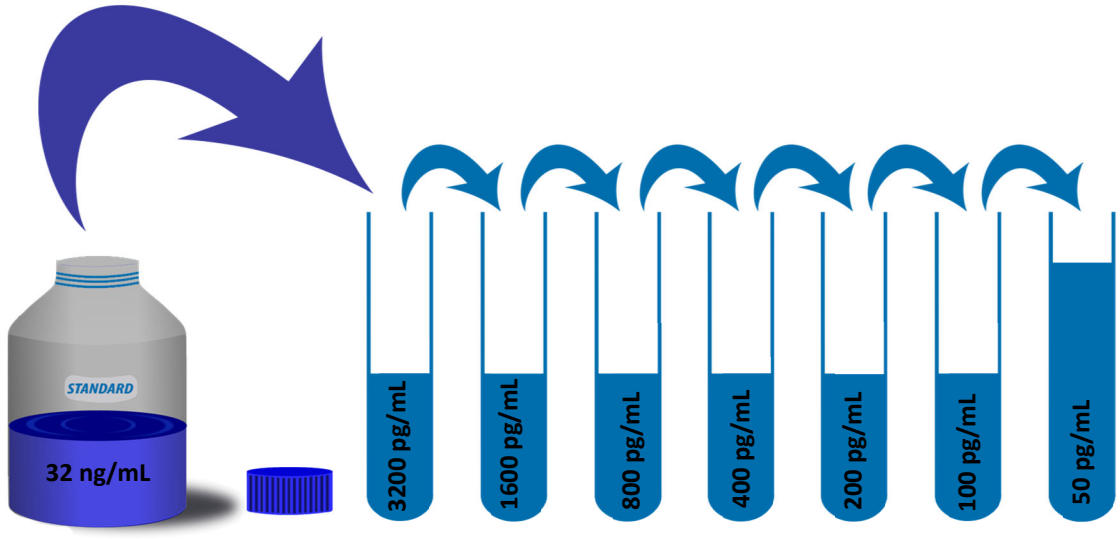
- For measuring Progesterone in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM. The assay has been validated using RPMI-1640.

All the samples must be used within 2 hours of preparation.

Standard preparation

- Prepare a 1:10 dilution of Progesterone Standard with Assay Buffer (mix 50 µL of standard with 450 µL of Assay Buffer), and label as the Standard No.7 (3200 pg/mL).
- The Progesterone Standard contains an organic solvent. Prerinse the pipette tip several times to ensure accurate delivery
- Make series of lower dilutions as described in the table.
- The Assay Buffer is used as the 0 pg/mL standard.

No.	*Concentration*	*Material needed*
Standard No.7	3200 pg/mL	50 µL Progesterone Standard + 450 µL Assay Buffer
Standard No.6	1600 pg/mL	250 µL Standard No.7 + 250 µL Assay Buffer
Standard No.5	800 pg/mL	250 µL Standard No.6 + 250 µL Assay Buffer
Standard No.4	400 pg/mL	250 µL Standard No.5 + 250 µL Assay Buffer
Standard No.3	200 pg/mL	250 µL Standard No.4 + 250 µL Assay Buffer
Standard No.2	100 pg/mL	250 µL Standard No.3 + 250 µL Assay Buffer
Standard No.1	50 pg/mL	250 µL Standard No.2 + 250 µL Assay Buffer
Standard No.0	0 pg/mL	250 µL Assay Buffer



All standard must be used within 2 hours of preparation

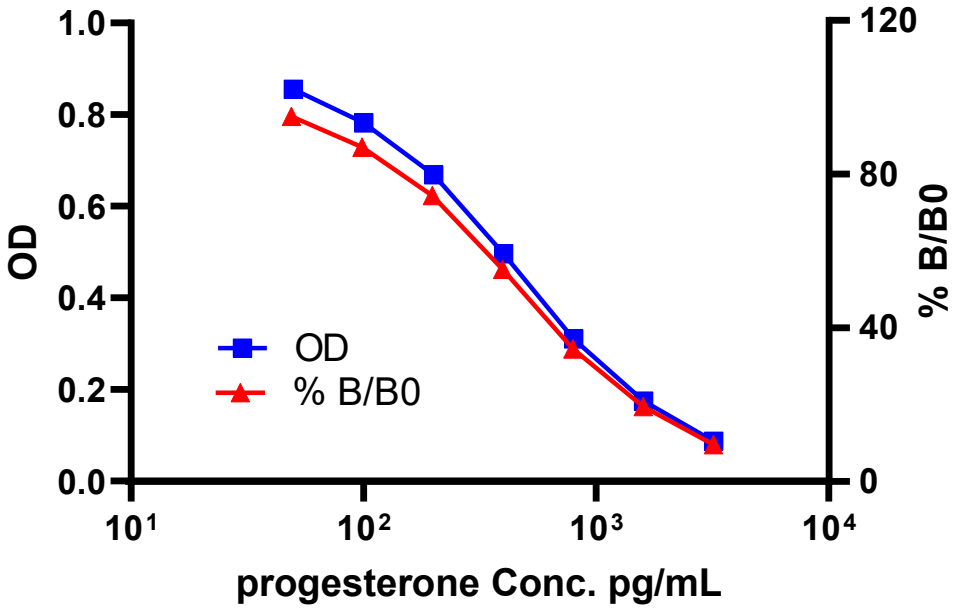
Assay Procedure

1. Pipette 50 µL of either samples or standards into duplicate wells in the plate.
2. Pipette 50 µL of Assay Buffer into duplicate wells of the Zero standard.
3. Pipette 75 µL of Assay Buffer into duplicate wells of the nonspecific binding (NSB).
4. Add 25 µL of Progesterone Conjugate to each well, using a repeater pipette.
5. Add 25 µL of Progesterone Antibody to each well except the NSB wells, using a repeater pipette.
6. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
7. Cover the plate with the plate sealer and shake for 2 hours at room temperature. If the plate is not shaken, signals will be approximately 45 % lower.
8. Aspirate the plate and wash each well 4 times with 300 µL Wash Buffer.
9. Tap the plate on clean absorbent towels to dry.
10. Add 100 µL of TMB Substrate to each well using a multichannel/repeater pipette.
11. Incubate at room temperature for 30 minutes without shaking.
12. Add 50 µL of Stop Solution to each well using a multichannel/repeater pipette.
13. Read the optical density at 450 nm.

Calculation

- Average the duplicate optical density (OD) readings for each standard and sample.
- Subtract the mean ODs of the NSB from all OD values
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader
- Calculate the % B/B0 ratio.
  - **Note:** B0 is the binding for the zero standard or the maximum binding well, which represents the maximum signal from enzyme captured by the specific antibody in competitive ELISA. All other standards and samples are expressed as a percentage of this value (% B/B0).
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.

  Conversion Factor: 100 pg/mL of Progesterone is equivalent to 318.0 pM



A typical standard curve of ZellX® Progesterone ELISA Assay kit

Run your own standard curves for calculation of results

Assay range

The detection limit of ZellX® Progesterone ELISA assay was determined as 52.9 pg/mL.

Sensitivity

The sensitivity of the ZellX® Progesterone ELISA assay was determined as 47.9 pg/mL.

Precision

Intra-Assay Precision (Precision within an assay): 3 human samples were tested 20 times in an assay.

Inter-Assay Precision (Precision between assays): 3 human samples were tested in duplicate on 14 different assays over multiple days.

Item	*% CV*
Intra assay	3.5, 5.1, 3.1
Inter assay	5.9, 7.0, 4.1

Cross Reactivity

The following cross reactants were tested in the assay and calculated at the 50 % binding point.

Steroid	*Cross Reactivity (%)*
Progesterone	100
3α-hydroxy-Progesterone	188
3β-hydroxy-Progesterone	172
11α-hydroxy-Progesterone	147
11β-hydroxy-Progesterone	2.7
5α-dihydroProgesterone	7.0
Pregnenolone	5.9
Corticosterone	< 0.1
Androstenedione	< 0.1

References

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