ab285257 – Progesterone ELISA Kit

For the quantitative determination of Progesterone.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
https://www.abcam.com/ab285257

Storage and Stability

On receipt entire assay kit should be stored at 4°C. Upon opening, use kit within 6 months.

Materials Supplied

Item	Quantity	Storage Condition
Goat anti-Mouse IgG Plate	8 x 12 strips	4°C
Progesterone Alkaline Phosphatase Conjugate	5 ml	4°C
Progesterone Monoclonal Ab	5 ml	4°C
Assay Buffer	27 ml	4°C
Wash Buffer Concentrate	27 ml	4°C
Progesterone Standard (100 ng/ml, in ethanol)	0.5 ml	4°C
pNpp Substrate	20 ml	4°C
Stop Solution	5 ml	4°C
Steroid Displacement Reagent	1 ml	4°C
Plate Sealer	1 unit	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 405 nm, preferably with correction between 570 and 590 nm
- Absorbent paper
- Adjustable pipettes and pipette tips
- Microplate shaker

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Wash Buffer: Prepare Wash Buffer by diluting 10 mL of the concentrate supplied with 190 mL of deionized water to make 200mL of Wash Buffer. This can be stored at room temperature until the kit expiration or for 3 months, whichever is earlier.

Warning and Precautions:

- Stop Solution is a solution of trisodium phosphate. This solution is caustic: care should be taken in use.
- The activity of the alkaline phosphatase conjugate is dependent on the presence of Mg2+ and Zn2+ ions. The activity of the conjugate is affected by concentrations of chelators (>10 mM) such as EDTA and EGTA.
- The performance of this kit has been tested using a variety of samples; however, it is possible that high levels of interfering substances may cause variation in assay results.
- The Progesterone Standard is supplied in ethanolic buffer at a pH optimized to maintain Progesterone integrity. Care should be taken when handling this material because of the known and unknown effects of steroids on biological tissue.

Sample Handling:

- This kit is compatible with Progesterone samples in a wide range of matrices.
- Samples diluted sufficiently into Assay Buffer can be read directly from the standard curve.
- Steroid Displacement Reagent should be added to serum, plasma and other samples containing steroid binding proteins. It will disassociate steroid from the binding protein allowing it to be detected by the assay. Treat appropriate samples with Steroid Displacement Reagent in the following manner: add one part of Steroid Displacement Reagent to 99 parts of sample. Once the Steroid Displacement Reagent has been added to the neat samples, briefly vortex the sample, allow it to sit for approximately 5 min. and then proceed with sample dilution. The Steroid Displacement Reagent needs to be added prior to any subsequent dilutions of the sample.
- Samples in the majority of tissue culture media can also be read in the assay after being diluted, provided the standards have been diluted into the tissue culture media instead of Assay Buffer.
- There will be a small change in the binding associated with running the standards and samples in media. Users should only use standard curves generated in media or buffer to calculate concentrations of Progesterone in the appropriate matrix.

Δ Note: *Tissue culture media may need to be diluted in the Assay Buffer in order to avoid matrix interference. If samples require further dilution in Assay Buffer, the standard curve must be prepared in a like manner (e.g., 1:10 dilution of tissue culture media in Assay Buffer).*
Δ Note: *Recommended dilution for tissue culture media (1:10), human serum and saliva (1:10). Please note, however, the end user must verify that the recommended dilutions are appropriate for their samples.*
Δ Note: *Samples containing mouse IgG may interfere with the assay.*
Δ Note: *Some samples may have very low levels of Progesterone present and extraction may be necessary for accurate measurement.*
Extraction procedure:

1. *Prepare 1 ng/mL Progesterone Standard solution for determination of extraction efficiency in sample matrix. This solution can be made by diluting 5 µL of the supplied Standard with 500 µL of ethanol (ACS grade or equivalent).*
2. *Add sufficient Progesterone to a sample matrix for determination of extraction efficiency. For a typical experiment based on a one mL sample starting volume, add 25-50 pg/mL of the 1 ng/mL Progesterone solution.*
3. *In a fume hood add 1 mL of Diethyl Ether (ACS Grade) for every mL of sample. Put Stopper and shake sample.*
4. *Allow layers to separate. Carefully pipet off the top ether layer and place in a clean test tube.*
5. *Repeat steps 2 and 3 twice more, combining the ether layers.*
6. *Evaporate the ether to dryness under nitrogen. Dissolve the extracted Progesterone with at least 250 µL of Assay Buffer. Vortex well, then allow to sit for 5 min. at room temperature. Repeat vortex step twice.*
7. *Run the reconstituted samples in the assay immediately. If analysis is to be delayed; store evaporated samples, desiccated, at or below -20°C.*

Assay Protocol

- A Standard Curve must be run with each assay.
- Bring all Buffer(s) to RT 30 minutes prior to the assay.
- All standards and samples should be run in duplicate.

1. Place the desired no. of coated strips into the holder. Keep any unused wells with the desiccant back into the pouch, seal Ziploc and store at 4°C.
2. Progesterone Standard: Allow the 100 ng/mL Progesterone Standard solution to warm to room temperature. Label six 12 x 75 mm glass tubes 1 through 6. Pipet 2 mL of standard diluent (Assay Buffer or Tissue Culture Media) into tube 1. Pipet 500 µL of standard diluent into tubes 2 through 6. Remove 10 µL of diluent from tube 1. Add 10 µL of the 100 ng/mL standard to tube 1. Vortex thoroughly. Add 500 µL of tube 1 to tube 2 and vortex thoroughly. Add 500 µL of tube 2 to tube 3 and vortex. Continue this for tubes 4 through 6. The concentration of Progesterone in tubes 1 through 6 will be 500, 250, 125, 62.5, 31.25, and 15.62 pg/mL, respectively.
3. Pipet 100 µL of standard diluent (Assay Buffer or Tissue Culture Media) into the NSB (non-specific binding) and the Bo (binding for 0 pg/mL Standard) wells.
4. Pipet 100 µL of Standards (1-6) and samples into the appropriate wells.
5. Pipet 50 µL of Assay Buffer into the NSB wells.
6. Pipet 50 µL of Progesterone Alkaline Phosphatase Conjugate into each well, except the Total Activity (TA) and Blank wells.
7. Pipet 50 µL of Progesterone Antibody Solution into each well, except the Blank, TA and NSB wells.

Δ Note: *Every well used should be Green in color except the NSB wells which should be Blue. The Blank and TA wells are empty at this point and have no color.*

8. Tap the plate gently to mix. Incubate at room temperature on a plate shaker for 2 hours at ~500 rpm. The plate may be covered with the plate sealer provided, if so desired.
9. At the end of the first incubation, empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 2 more times for a total of 3 Washes. After the final wash, empty or aspirate the wells and firmly tap the plate dry on a lint free paper towel to remove any remaining wash buffer.
10. Add 5 µL of the Progesterone Alkaline Phosphatase Conjugate to the TA wells.
11. Add 200 µL of the pNpp Substrate solution to every well. Incubate at room temperature for 45 min. without shaking.
12. Add 50 µL of Stop Solution to every well. This stops the reaction, and the plate should be read immediately.
13. Blank the plate reader against the Blank wells, read the optical density at 405 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all readings.

Calculation:

- Several options are available for the calculation of the concentration of Progesterone in samples. We recommend that the data be handled by an immunoassay software package utilizing a four-parameter logistic curve-fitting program. Such software is often supplied by plate reader manufacturers.
- Samples with concentrations outside of the standard curve range will need to be re-analysed using a different dilution. If this sort of data reduction software is not readily available, the concentration of Progesterone can be calculated as follows:

Calculate the average net OD bound for each standard and sample by subtracting the average NSB OD from the average OD bound:

Average Net OD = (Average OD)-(NSB OD)

Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo):

Percent Bound = (Net OD/Net Bo OD) x 100

- Graph the data points and the best-fit line through the points. The concentration of Progesterone in the unknowns can be determined by interpolation.

Technical Support

For all technical or commercial enquiries please go to:
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