Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Allopregnanolone ELISA Kit

08/17

(Catalog # E4346-100, 100 assays, Store at -20°C)

I. Introduction:

Allopregnanolone (3α-hydroxy-5α-pregnan-20-one) is a neurosteroid present in the blood and the brain. Allopregnanolone is made from progesterone which is converted into 5α-dihydroprogesterone by 5α-reductase type I. Allopregnanolone aids neurogenesis and has been found to reverse neuron proliferative deficit and cognitive deficits in a mouse model of Alzheimer's disease3. Allopregnanolone has also been shown to restore functionality in a mouse model of Parkinson's Disease4 and to improve behavioral problems in post-traumatic stress disorder. BioVision's Allopregnanolone ELISA kit is a competitive ELISA assay for the quantitative measurement of Allopregnanolone in extracted serum and plasma, or in urine, extracted dried fecal samples, and tissue culture media samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Allopregnanolone.
Detection Range: 10000 – 156.25 pg/ml
Sensitivity: < 129.7 pg/ml
Detection Limit: 65.9 pg/ml

III. Specificity:

Universal

IV. Sample Type:

Urine, tissue culture samples, extracted serum and plasma, or extracted dried fecal samples

V. Kit Contents:

Components	E4346-100	Part No.
Micro ELISA Plate	8 X 12 strips	E4346-100-1
Standard	125 µl	E4346-100-2
Allopregnanolone Antibody	3 ml	E4346-100-3
Allopregnanolone Conjugate	3 ml	E4346-100-4
Assay Buffer Concentrate (5X)	28 ml	E4346-100-5
Wash Buffer Concentrate (20X)	30 ml	E4346-100-6
TMB Substrate	11 ml	E4346-100-7
Stop Solution	5 ml	E4346-100-8
Plate Sealer	1	E4346-100-9

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Ethyl acetate or ethanol for serum, plasma or fecal extracts
- Speedvac for evaporation of ethanol or ethyl acetates
- Precision pipettes with disposable tips

VII. Storage and Handling:

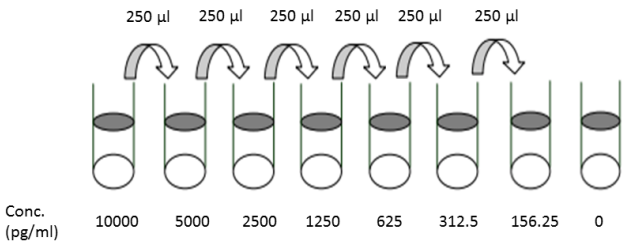
The entire kit may be stored at -20°C for up to 6 months. Avoid freeze-thaw cycles.

VIII. Reagent Preparation:

Note: Prepare reagents within 30 minutes before the experiment.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Assay Buffer:** Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.
2. **Wash Buffer**: Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable for 3 months at room temperature.
3. **Standard Preparation:**

- Add 50 µl of the Allopregnanolone stock solution to 450 µl of Assay Buffer (tube #1) and vortex completely.
- Prepare 4 vials of standards (tube #2-6) by adding 250 µl of the above stock solution in 250 µl of Assay Buffer. Perform 3-fold serial dilutions of the top standards to make the standard curve within the range of this assay.
- Suggested standard points are: 10000, 5000, 2500, 1250, 625, 312.5, 156.25 pg/ml.
- Use all Standards within 2 hours of preparation.



4. **Sample Preparation:**

FOR RESEARCH USE ONLY! Not to be used on humans.

Note: Use all Samples within 2 Hours of preparation, or stored at ≤ -20°C until assaying. Avoid multiple freeze-thaw cycles.

- **Extracted serum and plasma:** Add diethyl ether or ethyl acetate to serum or plasma samples at a 5:1 (v/v) solvent sample ratio. Mix solutions by vortexing for 2 minutes. Allow layers to separate for 5 minutes. Freeze samples in a dry ice/ethanol bath and pipet off the solvent solution from the top of the sample into a clean tube. Repeat steps 1-3 for maximum extraction efficiency, combining the solvent solutions. Dry pooled solvent extracts down in a speedvac for 2-3 hrs. If samples need to be stored they should be kept at -20°C. Redissolve samples at room temperature in diluted Assay Buffer. A minimum of 125 µl of Assay Buffer is recommended for reconstitution to allow for duplicate sample measurement.
- **Urine**: Urine samples should be diluted at least ≥ 1:4 in diluted Assay Buffer.
- **Tissue Culture Media:** For measuring allopregnanolone in tissue culture media (TCM), samples must be diluted in Assay Buffer. The end-user should determine the appropriate dilution for the media samples prior to running their samples.
- End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.

IX. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VIII.
2. Pipet 50 µl of samples or standards into wells in the plate. Pipet 75 µl of Assay Buffer into the non-specific binding (NSB) wells.
3. Add 25 µl of the Allopregnanolone Conjugate to each well. Add 25 µl of the Allopregnanolone Antibody to each well, except the NSB wells.
4. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 2 hour. If the plate is not shaken, signals bound will be approximately 45% lower.
5. Aspirate the plate and wash each well 4 times with 300 µl wash buffer. Tap the plate dry on clean absorbent towels.
6. Add 100 µl of the TMB Substrate to each well. Incubate the plate at room temperature for 30 minutes.
7. Add 50 µl of the Stop Solution to each well.
8. Read the optical density at 450 nm within 15 minutes.

X. CALCULATION:

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean OD's for the non-specific binding well (NSB). The sample concentrations obtained, calculated from the $\%B/B_0$ curve, and should be multiplied by the dilution factor to obtain neat sample values.

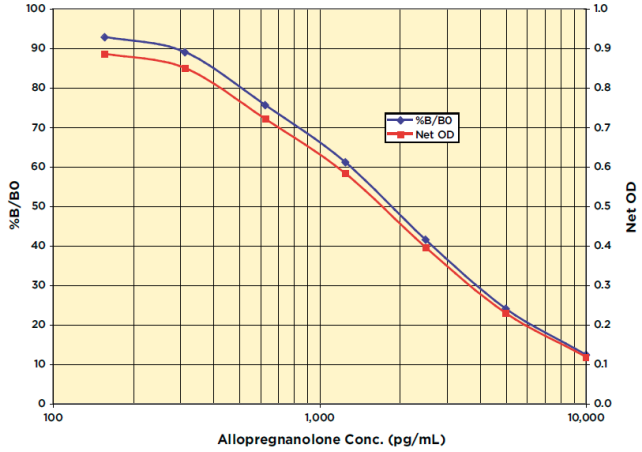


Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

XI. VALIDATION DATA:

Linearity: and Recovery Rate:

High Fecal Extract	Low Fecal Extract	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80%	20%	2,046.9	2,027.5	99.1%
60%	40%	1,625.1	1,580.4	97.2%
40%	60%	1,203.4	1,187.6	98.7%
20%	80%	781.7	764.3	97.8%
			Mean Recovery	**98.2%**

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Intra Assay:

Sample	Allopregnanolone Conc. (pg/mL)	%CV
1	3,576.5	3.7
2	1,768.8	4.6
3	545.7	12.9

Inter Assay Precision:

Sample	Allopregnanolone Conc. (pg/mL)	%CV
1	3,538.8	6.2
2	1,710.6	7.0
3	499.2	11.2

Cross Reactivity:

Steroid	Cross Reactivity (%)	Steroid	Cross Reactivity (%)
Allopregnanolone	100%	Estrone	< 0.08%
Tetrahydrodeoxycorticosterone (THDOC)	3.08%	Dihydrodeoxycorticosterone (DHDOC)	< 0.08%
Pregnanolone	2.19%	11α-hydroxyprogesterone	< 0.08%
Progesterone	0.12%	20α-hydroxyprogesterone	< 0.08%
Dihydrotestosterone	0.095%	Cortisone	< 0.08%
Tetrahydrocorticosterone	< 0.08%	Cortisol	< 0.08%
5α-dihydroprogesterone	< 0.08%	Estradiol	< 0.08%
Corticosterone	< 0.08%	Testosterone	< 0.08%

XII. RELATED PRODUCTS:

- Progesterone receptor (PGR) (Human) ELISA Kit (Cat. No. K4270)
- Progesterone (human) ELISA Kit (Cat. No. K7414)
- Progesterone ELISA Kit (Cat. No. K7416)
- Progesterone (Cat. No. K2913)
- Cortisol (human/mouse/rat) ELISA Kit (Cat. No. 7430)
- Corticosterone (CORT) ELISA Kit (Cat. No. K4222)
- 17-Hydroxyprogesterone ELISA Kit (E4345-100)