ab205928 – Arg^{8}-Vasopressin ELISA Kit

Instructions for Use

For quantitative detection of Arg^{8}-Vasopressin in tissue culture media.

This product is for research use only and is not intended for diagnostic use.

Version 5a Last Updated 29 October 2021

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1. BACKGROUND

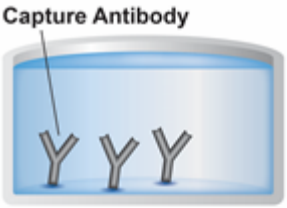
Abcam's Arg^{8}-Vasopressin *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Arg^{8}-Vasopressin in plasma, serum, and tissue culture media.

A goat anti-rabbit IgG antibody has been precoated onto 96-well plates. Standards or test samples are added to the wells, along with a biotin conjugated- Arg^{8}-Vasopressin antigen and a polyclonal rabbit antibody specific to Arg^{8}-Vasopressin. After incubation the excess reagents are washed away. TMB substrate is added and after a short incubation the enzyme reaction is stopped and the yellow color generated is read at 450 nm. The intensity of the yellow coloration is inversely proportional to the amount of Arg^{8}-Vasopressin captured in the plate.

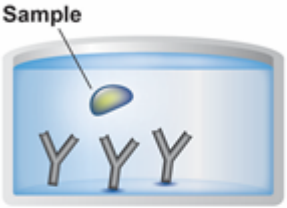
Arginine Vasopressin (AVP) is a 9 amino acid peptide with a 6-member disulfide ring. It is structurally related to oxytocin differingin 2 amino acids. It is synthesized in the hypothalamus supraoptic and paraventricular nuclei. It is stored in the posterior pituitary for release. AVP has powerful antidiuretic action and is also known as antidiuretic hormone (ADH). It acts upon the collecting tubule of the kidney increasing permeability to water and urea. It also has neurotransmitter and peripheral humoral functions.

AVP has been shown to be released upon both osmotic and non-osmotic stimuli and its release into peripheral blood causes effects upon a number of factors, including emotional stress, posture, blood volume, and temperature. Alcohol appears to inhibit AVP secretion. Serum AVP measurement is used clinically for studies involving diabetes insipidus, syndrome of inappropriate ADH secretion (SIADH), ectopic AVP production and pyschogenic water intoxication.

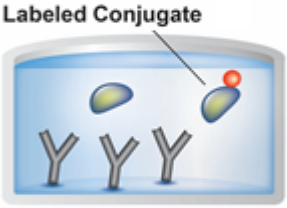
2. ASSAY SUMMARY



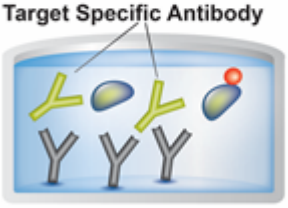
Prepare all reagents and samples as instructed.



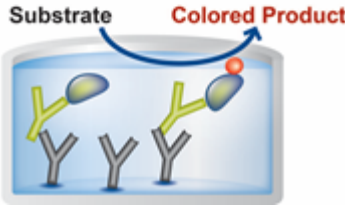
Add standards and samples to appropriate wells.



Add prepared labeled Biotin-conjugate to appropriate wells.



Add Vasopressin antibody to appropriate wells. Incubate at room temperature.



Add TMB substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

- Some kit components contain azide, which may react with lead or copper plumbing. When disposing of reagents always flush with large volumes of water to prevent azide build-up
- Stop Solution is a solution of trisodium phosphate. This solution is caustic; care should be taken in use
- We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results
- The Vasopressin Standard provided, is supplied in ethanolic buffer at a pH optimized to maintain Vasopressin integrity. Care should be taken handling this material because of the known and unknown effects of Vasopressin.

4. STORAGE AND STABILITY

Store kit at +2-8°C immediately upon receipt, apart from the Vasopressin Standard, which should be stored at -20°C. Avoid multiple freeze-thaw cycles.

Refer to list of materials supplied for storage conditions of individual components.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Goat anti-rabbit IgG Microplate (12 x 8 wells)	96 Wells	+2-8ºC
Vasopressin Biotin Conjugate	5 mL	+2-8ºC
Vasopressin polyclonal rabbit Antibody	5 mL	+2-8ºC
Vasopressin Standard	500 µL	-20ºC
Assay Buffer	27 mL	+2-8ºC
20X Wash Buffer Concentrate	27 mL	+2-8ºC
SA-HRP	20 mL	+2-8ºC
TMB Substrate	20 mL	+2-8ºC
Stop Solution	10 mL	+2-8ºC
Plate Sealer	1 Unit	+2-8ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Standard microplate reader - capable of reading at 450 nm, preferably with correction between 570 and 590 nm.
- Automated plate washer (optional).
- Deionized or distilled water.
- Precision pipets for volumes between 5 µL and 1,000 µL.
- Repeater pipets for dispensing 50 µL and 200 µL.
- Disposable beaker for diluting buffer concentrates.
- Graduated cylinders.
- Lint-free paper for blotting.
- A microplate shaker.
- Nitrogen gas (required for the suggested extraction protocol).
- Acetone (required for the suggested extraction protocol).
- Petroleum Ether (required for the suggested extraction protocol).
- Butanol (optional for the suggested extraction protocol).
- Diisopropyl ether (optional for the suggested extraction protocol).

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- Standards can be made up in either glass or plastic tubes.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the well to avoid contamination.
- This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at +2-8°C in the sealed bag provided. The wells should be used in the frame provided.
- Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18 - 25°C) prior to use.

9.1 **Arg^{8}-Vasopressin Biotin Conjugate**

Allow the Vasopressin Biotin Conjugate to equilibrate to room temperature.

9.2 **1X Wash Buffer**

Prepare the 1X Wash Buffer by diluting 5 mL of the 20X Wash Buffer Concentrate in 95 mL of deionized water. Mix thoroughly and gently. This can be stored at room temperature until the kit expiration date, or for 3 months, whichever is earlier.

10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use. Diluted standards should be used within 60 minutes of preparation.

10.1 Allow the 10,000 pg/mL Vasopressin Stock Standard solution to equilibrate to room temperature. The standard solution should be stored at -20°C. Avoid repeated freeze-thaw cycles.

10.2 Label eight tubes with numbers 1 – 7 and one with B_O.

10.3 Prepare a 1,000 pg/mL **Standard 1** by adding 100 µL of the 10,000 pg/mL Stock to 900 µL of Assay Buffer into tube 1. Mix thoroughly and gently.

10.4 Add 600 µL of the Assay Buffer into tubes numbers 2- 7.

10.5 Prepare **Standard 2** by transferring 400 µL from Standard 1 to tube 2. Mix thoroughly and gently.

10.6 Prepare **Standard 3** by transferring 400 µL from Standard 2 to tube 3. Mix thoroughly and gently.

10.7 Using the table below as a guide, repeat for tubes 4 to 7 and B_O.

Standard	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Stock	100	900	10,000	1,000
2	Standard 1	400	600	1,000	400
3	Standard 2	400	600	400	160
4	Standard 3	400	600	160	64
5	Standard 4	400	600	64	25.6
6	Standard 5	400	600	25.6	10.24
7	Standard 6	400	600	10.24	4.10
8 (Bo)	None	-	600	-	0

11. SAMPLE COLLECTION AND STORAGE

- The Arg^{8}-Vasopressin ELISA kit is compatible with Vasopressin samples in a number of matrices. Vasopressin samples should be reconstituted in kit Assay Buffer for extracted serum and plasma samples and diluted into Assay Buffer for tissue culture medium samples. The end user should verify that the recommended dilutions are appropriate for their samples. Samples containing rabbit IgG may interfere with the assay.
- Due to the low endogenous levels of native Arg^{8}-Vasopressin extraction of samples is recommend, thereby concentrating them and allowing for accurate determinations of Arg^{8}-Vasopressin. An extraction protocol is outlined below. Because of the labile nature of Vasopressin several precautions are recommend in collecting and analyzing samples.
- Blood samples should be drawn into chilled EDTA (1 mg/mL blood) or serum tubes containing Aprotonin (500 KIU/mL of blood). Centrifuge the samples at 1,600 x g for 15 minutes at +2-8°C. Transfer the plasma or serum to a plastic tube and store at -70°C or lower for long term storage. Avoid repeated freeze/thaw cycles. The stability of some peptides is improved by the addition of a protease inhibitor cocktail to the sample before freezing.

Delipidation Procedure:

If samples are thought to be lipemic, the following procedure can be used to delipidate prior to extraction.

1. Prepare mixture of 40:60 butanol:diisopropyl ether. Vortex.
2. Add equal volume of butanol:diisopropyl ether to sample. Vortex.
3. Centrifuge at 8,000 x g for 5 minutes.
4. Remove top organic layer and discard. Measure aqueous layer and transfer to new tube.

Extraction Procedure:

1. Add 2x volume of ice cold acetone to sample. Vortex.
2. Centrifuge at 3,000 x g for 20 minutes.
3. Transfer supernatant to new tube.
4. Add 5x volume of ice cold petroleum ether. Vortex.
5. Centrifuge at 3,000 x g for 10 minutes.
6. Discard top ether layer. Carefully transfer remaining aqueous layer to glass tube and dry down under gas.
7. Reconstitute sample with Assay Buffer.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at +2-8°C
- For statistical reasons, it is recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

Recommended plate layout

A	Std 1	Std 1	Blank	Blank							
B	Std 2	Std 2	NSB*	NSB*							
C	Std 3	Std 3									
D	Std 4	Std 4									
E	Std 5	Std 5									
F	Std 6	Std 6									
G	Std 7	Std 7									
H	Std 8 (B0)	Std 8 (B0)									
	1	**2**	**3**	**4**	**5**	**7**	**8**	**9**	**10**	**11**	**12**

*NBS = Non-specific binding.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use**
- **It is recommended to assay all standards, controls and samples in duplicate**
- **Refer to the recommended plate layout in Section 12 before proceeding with the assay**

13.1 Add 100 µL of Assay Buffer into the NSB and the Bo (0 pg/mL Standard) wells.

13.2 Add 100 µL of Standards #1 through #7 into the appropriate wells.

13.3 Add 100 µL of the Samples into the appropriate wells.

13.4 Add an additional 50 µL of Assay Buffer into the NSB wells.

13.5 Add 50 µL of the blue Conjugate into each well, except the Blank wells.

13.6 Add 50 µL of the yellow Antibody into each well, except the Blank and NSB wells.

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

13.7 Tap the plate gently to mix. Seal the plate and incubate at +2-8°C for 18-24 hours.

13.8 Empty the contents of the plate and wash by adding full well volume (~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 on a lint free paper towel to remove any remaining wash buffer.

13.9 Add 200 µL of the SA-HRP to every well, except the Blank wells.

13.10 Seal the plate and incubate at room temperature on a plate shaker for 30 minutes at ~500 rpm*.Wash as above (step 13.8).

13.11 Add 200 µL of the TMB Substrate to every well.

13.12 Seal the plate and incubate at room temperature on a plate shaker for 30 minutes at ~500 rpm*.

13.13 Add 100 µL of Stop Solution to every well. This stops the reaction and the plate should be read immediately.

13.14 Blank the plate reader against the Blank wells, and read the optical density at **450 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.

*The actual speed of the plate shaker should be such that the liquid in the plate wells mixes thoroughly, but does not splash out of the well.

14. CALCULATIONS

A four parameter algorithm (4PL) provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted.

14.1 Calculate the average net optical density (OD) bound for each standard and sample by subtracting the average NSB OD from the average OD bound:

Average Net OD = Average Bound OD - Average NSB OD

14.2 Plot the Net OD versus the Concentration of Arg^8-Vasopressin for the standards. Sample concentrations of Arg^8-Vasopressin may be calculated by interpolation off the standard curve using Net OD values.

14.3 Alternatively, calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:

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

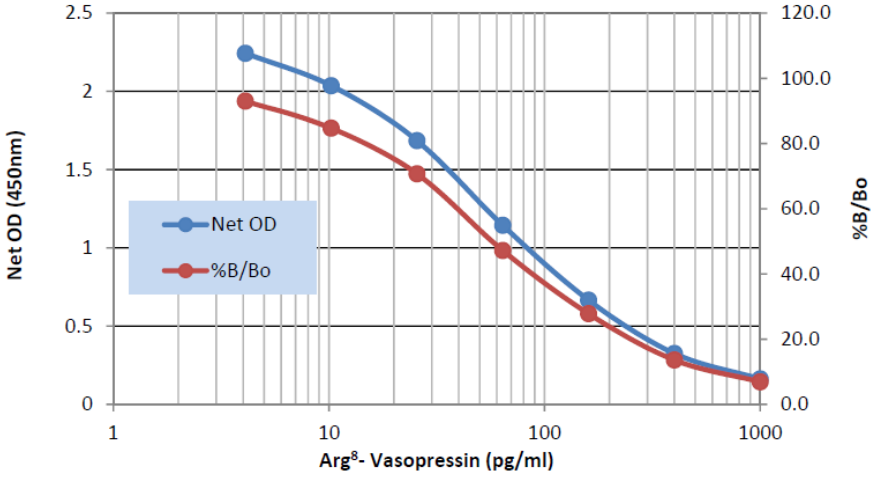
14.4 Plot Percent Bound versus Concentration of Arg^8-Vasopressin for the standards. The concentration of Arg^8-Vasopressin in the unknowns may then also be determined by interpolation off of the binding curve.

Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

TYPICAL STANDARD CURVE



Sample	Vasopressin (pg/mL)	Net Optical Density (450nm)	%B/Bo
Bo	0	2.419	-
Sample 1	1000	0.162	6.9
Sample 2	400	0.325	13.6
Sample 3	160	0.666	27.8
Sample 4	64	1.144	47.2
Sample 5	25.6	1.685	70.7
Sample 6	10.24	2.032	84.7
Sample 7	4.096	2.243	93.0
NSB	-	0.031	-
TA	-	0.679	-

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The sensitivity of the assay was determined by interpolation from the average of 9 separate standard curves run with replicate data points at each concentration. The sensitivity was determined at 2 standard deviations below the average net OD of 54 zero standard replicates (6 per standard curve). The sensitivity (limit of detection) of the assay is 2.84 pg/mL.

SAMPLE RECOVERY –

Vasopressin concentrations were measured in tissue culture media. Vasopressin was spiked into the undiluted media and measured neat or following dilution with Assay Buffer. Control spikes into Assay Buffer were also measured. The following results were obtained:

Sample Type	Average % Recovery	Recommended Dilution
Tissue Culture Media	116.2%	No dilution needed
Tissue Culture Media with 10% bovine serum	100.0%	1:2
Assay Buffer	111.4%	No dilution needed

LINEARITY OF DILUTION –

A buffer sample containing Vasopressin was serially diluted 5 times 1:2 in the kit Assay Buffer and measured in the assay.

The data was plotted graphically as expected Vasopressin concentration versus observed Vasopressin concentration. The line obtained had a slope of 1.0162 with a correlation coefficient of 0.9895.

Linearity

Dilution	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)
Neat	-	390.8	-
1:2	195.4	194.6	100%
1:4	97.7	105.1	108%
1:8	48.8	67.0	137%
1:16	24.4	27.9	114%
1:32	12.2	12.1	99%

PRECISION –

	Vasopressin (pg/mL)	Intra-Assay %CV
High	143.7	6.0
Medium	70.7	6.7
Low	32.1	14.3

	Vasopressin (pg/mL)	Inter-Assay %CV
High	136.2	8.6
Medium	66.4	6.4
Low	33.0	9.5

17. ASSAY SPECIFICITY

This kit detects both endogenous and recombinant Vasopressin.

CROSS REACTIVITY –

The % cross reactivity for each related compound was determined by running serial dilutions of each compound (10,000 pg/mL – 10 pg/mL) in the assay, fitting the resulting dose response curve to 4PL curve and determining the ED50. The ED50 of the standard curve was then

divided by the determined ED50 of the cross reactant and multiplied by 100.

Analyte	Cross Reactivity
Arg^8-Vasopressin	100 %
Lys^8 Vasopressin	9.8 %
Oxytocin	<0.001 %
TRH	<0.001 %
VIP	<0.001 %
Leu-Enkephalin	<0.001 %
Met-Enkephalin	<0.001 %
Mesotocine	<0.001 %
Cyclo-Somatostatin	<0.001 %
Vasotocin	4.8 %
Desmopressin	3.1 %
Ser^4 Ile^8 Oxytocin	<0.001 %

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

19. NOTES

For all technical and commercial enquires please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)