VASOPRESSIN Direct

RIA

This product is for research use only
It is not intended for use in diagnostic procedures

RK-VPD-U 100 tests

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ENGLISH

INTENDED USE

This double antibody radioimmuno-assay is designed for the quantitative *in vitro* direct measurement of **arginine vasopressin** (anti-diuretic hormone, ADH) in EDTA plasma (1-3). This product is for research use only. It is not intended for use in diagnostic procedures.

PRINCIPLE OF THE ASSAY

Immunoreactive vasopressin is measured with a double antibody radioimmunoassay according to a modified method of Glick and Kagan (4). Samples and calibrators are at first pre-incubated with the anti-vasopressin antibody for 24 hours. ^{125}I-vasopressin then competes with vasopressin present in samples and calibrators for the same antibody binding sites. After again 24 hour incubation, the solid-phase second antibody is added to the mixture, and the antibody-bound fraction is finally precipitated and counted.

REAGENTS SUPPLIED AND PREPARATION

Reagents	Quantity	Code	Reconstitution
Phosphate Buffer lyophilized Buffer	1 vial	B-VPD-PB	Reconstitute with 50 ml of deionized water
Antibody Dilution Buffer	1 vial 6 ml	B-VPD-DB	Ready to use
Antiserum lyophilized anti-vasopressin antibody	1 vial	B-VPD-AS	Reconstitute with 5 ml of Antibody Dilution Buffer
Tracer lyophilized ^{125}I-Vasopressin	1 vial	B-ADH-TR	Reconstitute with 11 ml of Phosphate Buffer
Calibrator[1)] Lyophilized synthetic arginine vasopressin in Phosphate Buffer	1 vial	B-VPD-CA	Reconstitute with 5 ml of deionized water
Controls Normal / High[2)] Arginine Vasopressin in a buffer matrix	2 vials	B-VPD-CONSET	Reconstitute with 5 ml of deionized water
Second Antibody Cellulose coated anti-rabbit antibody	1 vial 11 ml	B-AB2	Ready to use

Table 1

[1)] Reconstitution of the Calibrator results in a stock solution of 80 pg/ml arginine vasopressin [Arg 8]-vasopressin.

[2)] Lot specific amounts of arginine vasopressin in buffer matrix. Refer to the additional QC Data Sheet for exact concentrations.

STORAGE AND SHELF LIFE OF REAGENTS

Unopened Reagents	
The kit components are stable at 2-8°C. Do not use the kit beyond the expiration date printed on the labels. Do not freeze the Second Antibody.	
Opened / Reconstituted Reagents	
Phosphate Buffer	Stable for 2 months at 2-8°C
Antibody Dilution Buffer	Store at 2-8°C until expiration date.
Antiserum	Stable for 2 months at -20°C
Tracer	Store at -20°C Aliquot if repeated use is expected
Calibrator	Stable for 2 months at -20°C Aliquot if repeated use is expected
Controls	
Second Antibody	Store at 2-8°C (do not freeze)

Table 2

WARNINGS AND PRECAUTIONS

Radioactive Material: This kit contains radioactive material which does not exceed 56 kBq (1.5 µCi) of Iodine 125. The receipt, acquisition, possession, use and transfer are subject to the local regulations. Concerning the proper precautions for the handling and disposal of kit reagents, radioactive material, radioactive waste and specimens, we highly recommend first to consult the special local regulations of your country.

Reagents Containing Human Source Material: All kit reagents besides the second antibody (B-AB2) contain components of human origin. Each serum used in the preparation of the kit components was tested by a FDA-approved method and found negative for HBV surface antigen, HCV and HIV1/2 antibodies. Although those methods are highly accurate, there is no guarantee that this material cannot transmit Hepatitis or AIDS. Therefore, human specimens and kit components should be handled as if capable of transmitting infections. All reagents and samples containing human material should be handled in accordance with Good Laboratory Practice (GLP) using appropriate precautions.

MATERIALS REQUIRED BUT NOT PROVIDED

- 50 µl, 100 µl, 250 µl, 400 µl and 1000 µl precision pipettes with disposable tips.
- 5.0 ml and 10 ml volumetric pipettes.
- Beaker and cylinder necessary for the reconstitution of the phosphate buffer.
- Disposable polystyrene tubes for the preparation of the calibrator dilutions.
- Disposable conical polystyrene tubes to run the assay (e.g. Sarstedt # 57.477).
- Distilled or deionized water.
- Vortex mixer.
- Stir bar and magnetic stirrer.
- Centrifuge.
- Aspiration device.
- Gamma counter.

SPECIMEN COLLECTION AND STORAGE

Appropriate sample collection is essential to ensure accurate results of the vasopressin analysis. The procedure calls for true basal levels, the individual must be fasting for at least 12 hours and must stay recumbent, without any stress and in a quiet environment, for at least 1 hour prior to blood collection.

Collect blood (at least 2 ml) into an **EDTA venipuncture tube** (see below) and immediately place the sample on ice. Centrifuge at 2-8°C at 2000 g for 15 minutes within 10 minutes after blood collection, separate the plasma from the cells and freeze the specimen immediately in a plastic tube at ≤ -20°C if it will not be used immediately. The procedure calls for 400 µl of plasma per assay tube.

PROCEDURAL NOTES

- It is imperative to **use EDTA plasma ONLY** in order to inhibit metalloprotease activities. Heparin plasma cannot be used in the Vasopressin-direct assay and will interfere with the binding of the antibody.
- Use of **conical polystyrene tubes** is strongly recommended. During step 11 of the assay procedure, a more solid pellet will be achieved and the following aspiration of the supernatant can be done much easier.
- **Calibrator Dilution:** In order to obtain an entire standard curve, serial dilutions of the Calibrator are prepared as follows:
  - Label seven tubes A through G and Pipet 1.0 ml of phosphate buffer into tubes B through G.
  - Pipet 1.0 ml of the reconstituted Calibrator stock solution (80 pg/ml) into tubes A and B, vortex.
  - Transfer 1.0 ml from tube B to tube C, vortex.
  - Continue to transfer 1.0 ml from each tube until dilution series is completed.

  The corresponding concentrations of arginine vasopressin will be:

A 80 pg/ml (73.6 pmol/l)
B 40 pg/ml (36.8 pmol/l)
C 20 pg/ml (18.4 pmol/l)
D 10 pg/ml (9.2 pmol/l)
E 5.0 pg/ml (4.6 pmol/l)
F 2.5 pg/ml (2.3 pmol/l)
G 1.25 pg/ml (1.1 pmol/l)

ASSAY PROCEDURE

Allow all reagents for steps 1-4 to come to room temperature (18-28°C) prior to use.

1. Label 10 polystyrene tubes in duplicate: A to G (Calibrator), NSB (blank), MB (maximum binding) and T (total activity). Label additional polystyrene tubes in duplicate for samples and controls.

2a. Pipet 700 µl of phosphate buffer into the NSB tubes and 650 µl into the MB tubes.

2b. Add 250 µl of phosphate buffer into the remaining tubes, except for T tubes.

3a. Pipet 400 µl of each Calibrator (from A to G) into the corresponding tubes.

3b. Pipet 400 µl of each sample and Control into the corresponding tubes.

4. Add 50 µl of the reconstituted vasopressin antiserum to all tubes except the NSB and T tubes. Vortex.
5. Incubate all tubes for 24± 3 hours at 18-28°C.
6. Add 100 µl of the reconstituted vasopressin tracer to all tubes. Vortex. Remove the T tubes for counting at step 12. They will not require any further processing.
7. Incubate for 24± 3 hours at 18-28°C.

8a. Invert the solid phase second antibody bottle several times, add a stir bar and place the bottle on a magnetic stirrer.

8b. While stirring the second antibody suspension continuously, add 100 µl of the suspension to all assay tubes (except the T tubes). Vortex.

9. Incubate for 20±1 minutes at 18-28°C. **Do not re-suspend the precipitate**.
10. Add 1 ml of deionized water to each tube (except the T tubes).
11. Centrifuge for 5 minutes 1000 x g. Aspirate the supernatant **(except the T tubes)** and retain the precipitates for counting.
12. Count all tubes for 1 minute in a gamma counter.

RESULTS

- Record the cpm for all tubes (T, NSB, MB, Calibrators A-G samples and Controls) and calculate the mean cpm for each pair of tubes.
- Subtract the mean assay blank (NSB tubes) from the respective mean of each pair of tubes:

  Net cpm = $cpm_{Average}$ - $cpm_{Average\ NSB}$

- Calculate the binding of each pair of tubes as a percent of maximum binding (MB tubes), with the NSB-corrected cpm of the MB tubes taken as 100%.

$$B/B_0(\%) = \text{percent bound} = \frac{\text{net cpm}}{\text{net MB cpm}} \times 100$$

- Prepare a lin/log graph paper and plot the percent bound on the vertical axis against the vasopressin concentration (pg/ml) on the horizontal axis for each of the Calibrators. Draw the best fitting curve or calculate the standard curve using a spline smoothed fitting algorithm.
- Determine Vasopressin concentrations for samples and Controls from this standard curve. Alternative data reduction methods are equally acceptable.

To get the pmol/L concentrations of the results, multiply the pg/ml values by a factor of 0.92.

Standardization: The BÜHLMANN [Arg 8]-vasopressin Calibrator was calibrated against WHO reference preparation 77/501.

See Table 3 and Figure 1 for examples of results and standard curves. These results and standard curves are for demonstration purposes only. A standard curve must be generated for each set of samples to be assayed.

QUALITY CONTROL

A thorough understanding of this instruction for use is necessary for the successful use of the product. Reliable results will be obtained only by using precise laboratory techniques (current GLP guidelines) and accurately following this instruction for use.

The accuracy of each actual calibrator lot is calibrated against WHO reference preparation 77/501.

The reproducibility of standard curve parameters and control values should be within established limits of laboratory acceptability. The confidence limits for the Controls are lot-specific and printed on the QC Data Sheet added to the kit.

If the precision of the assay does not correlate with the established limits and repetition excludes errors in technique, check the following issues: i) pipetting, temperature controlling and timing devices ii) expiration dates of reagents iii) storage and incubation conditions iv) purity of water.

LIMITATIONS AND TROUBLE SHOOTING

- Samples that are not properly collected and handled may cause inaccurate arginine vasopressin results (see Specimen Collection). Freezing plasma samples immediately will preserve the integrity of the vasopressin concentration at the time of sampling.
- If the initial concentration of an unknown sample reads greater than the highest calibrator, the sample should be diluted with phosphate buffer and tested again according to the assay procedure.
- Counting time should be sufficient to prevent statistical counting error: e.g., accumulation of 2000 cpm will result in 5% counting error, 10000 cpm in 1% counting error.

PERFORMANCE CHARACTERISTICS

Intra-Assay Precision (Within-Run): 6.0%. The intra-assay precision was calculated from the results of 10 pairs of values from each sample in a single run. The values are presented in Table 44.

Inter-Assay Precision (Run-to-Run): 9.9%. The inter-assay precision was calculated from the results of 20 pairs of values from two EDTA plasma samples in 20 different runs. The values are presented in Table 5.

Analytical sensitivity: 0.75 pg/ml (0.82 pmol/l). The analytical sensitivity of the Vasopressin Direct RIA was calculated by subtracting two standard deviations of averaged Zero Calibrator duplicates from the counts at maximum binding.

Functional Sensitivity: 1.3 pg/ml (1.2 pmol/l). The functional least detectable dose (FLDD) of the assay was calculated with a cut-off of the intra assay CV = 10%.

Spiking Recovery: 101.8 %. An EDTA plasma sample was spiked with increasing amounts of synthetic arginine-vasopressin and analyzed according to the assay procedure. The values are presented in Table 6.

Specificity: The following cross-reactions of the vasopressin antiserum were determined at 50% binding:

Arginine vasopressin	100.0	%
Lysine vasopressin*	**0.25**	**%**
Desmopressin (DDAVP)	0.0085	%
Oxytocin	0.001	%
Vasotocin	0.001	%

* In pigs and hippopotamus the arginine at position eight is replaced by lysine

Method comparison: The VASOPRESSIN-DIRECT RIA has been compared with the Bühlmann VASOPRESSIN RIA (RK-AR1; column extraction). Results obtained from 28 samples (EDTA plasma) show an excellent correlation (see Figure 2).

Table 3: **Example of Results**

Incubation temperature at BÜHLMANN: 20°C.
Data reduction: Multicalc Software (PerkinElmer), spline smoothed option.

	cpm	B/T [%]	B/B_0 [%]	Conc [pg/ml]	CV [%]
Total	31445	100.0			
Total	31261	100.0			
Total Avg.	**31353**	100.0			0.4
NSB	768	2.45			
NSB	701	2.25			
NSB Avg.	**736**	**2.35**			6.1
MB	9863	31.46	100		
MB	9921	31.65	100		
MB	10428	33.26	100		
MB	10083	32.16	100		
MB Avg.	**10074**	**32.13**	**100**		2.5
Calibrator G	9442	30.12	93.2	1.25	
Calibrator G	9444	30.12	93.3	1.25	
Calibrator G Avg.	**9443**	**30.12**	**93.2**	**1.25**	0.0
Calibrator F	9187	29.3	90.5	2.5	
Calibrator F	9215	29.39	90.8	2.5	
Calibrator F Avg.	**9201**	**29.35**	**90.7**	**2.5**	0.2
Calibrator E	8474	27.03	82.9	5.0	
Calibrator E	8440	26.92	82.5	5.0	
Calibrator E Avg.	**8457**	**26.97**	**82.7**	**5.0**	0.3
Calibrator D	6789	21.65	64.8	10.0	
Calibrator D	6819	21.75	65.1	10.0	
Calibrator D Avg.	**6804**	**21.70**	**65.0**	**10.0**	0.3
Calibrator C	5621	17.93	52.3	20.0	
Calibrator C	5681	18.12	53.0	20.0	
Calibrator C Avg.	**5651**	**18.02**	**52.6**	**20.0**	0.8
Calibrator B	4173	13.31	36.8	40.0	
Calibrator B	4319	13.78	38.4	40.0	
Calibrator B Avg.	**4246**	**13.54**	**37.6**	**40.0**	2.4
Calibrator A	2877	9.18	22.9	80.0	
Calibrator A	2909	9.28	23.3	80.0	
Calibrator A Avg.	**2893**	**9.23**	**23.1**	**80.0**	0.8
Control NORMAL	9036		88.9	2.94	
Control NORMAL	9009		88.6	3.02	
Control NORM. Avg	**9023**		**88.7**	**2.98**	2.0
Control **High**	6743		64.3	11.5	
Control High	6817		65.1	11.1	
Control High Avg.	**6780**		**64.7**	**11.3**	2.6

ED-20 = 92.7 pg/ml ED-50 = 22.4 pg/ml ED-80 = 5.42 pg/ml

Figure 1: **Example of a standard curve**

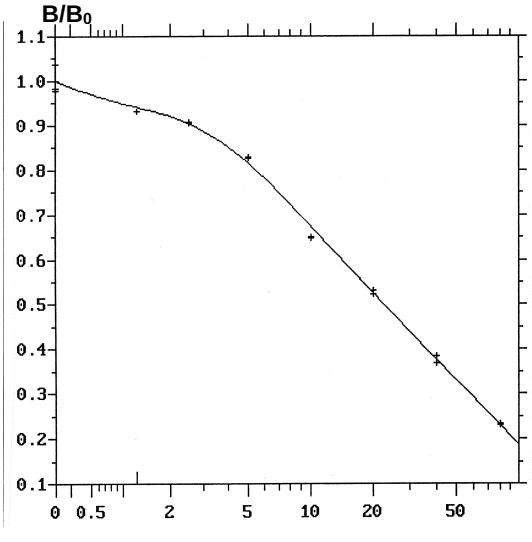


Table 4: **Intra-Assay Precision (Within-Run)**

Sample	Mean [pg/ml]	SD [pg/ml]	CV [%]
Plasma 1	4.0	0.39	9.5
Plasma 2	6.7	0.42	6.2
Plasma 3	19.1	0.44	2.3
Mean			6.0

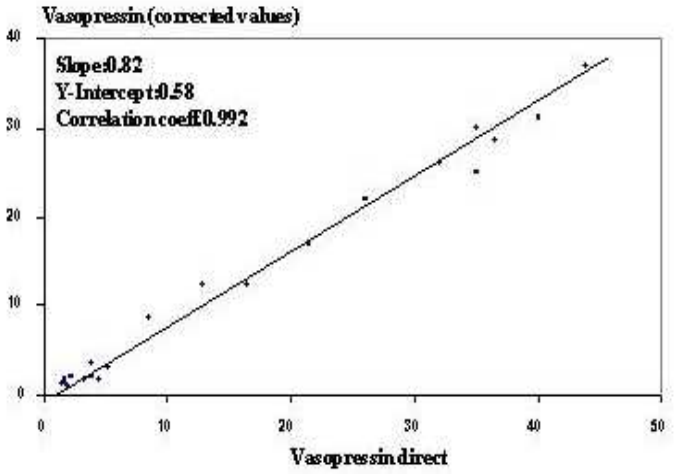
Table 5: **Inter-Assay Precision (Run-to-Run)**

Sample	Mean [pg/ml]	SD [pg/ml]	CV [%]
Plasma 4	1.78	0.23	13.0
Plasma 5	12.27	0.83	6.8
Mean			9.9

Table 6: **Spiking Recovery**

Sample	basic value [pg/ml]	spiked with [pg/ml]	Calculated [pg/ml]	Observed [pg/ml]	Recovery [%]
Plasma 6	2.0	2.6	4.6	5.0	109
	2.0	7.8	9.8	10.1	103
	2.0	23.3	25.3	26.4	104
	2.0	70.0	72.0	65.7	91
Mean					101.8

Figure 2: **Method Comparison RK-VPD vs. RK-AR1**



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RADIOIMMUNOASSAY PROCEDURE

Polystyrene tubes in duplicate	Phosph. Buffer (µl)	Standard, Control, Sample (µl)	Antiserum (µl)		Tracer (µl)		Second Antibody (µl)	
Total	--	--	--		100		--	**Vortex and incubate for 20 minutes (±1 minute) at 18-28°C**
NSB	700	--	--		100		100	
MB	650	--	50		100		100	
Std A 80 pg/ml	250	400	50	**Vortex and incubate at 18-28°C for 24 hours (± 3 h)**	100	**Vortex and incubate at 18-28°C for 24 hours (± 3 h)**	100	
Std B 40 pg/ml	250	400	50		100		100	**Add 1ml of deionized water (except T tubes) and centrifuge for 5 minutes at 2-8°C and 1000 x g**
Std C 20 pg/ml	250	400	50		100		100	
Std D 10 pg/ml	250	400	50		100		100	
Std E 5 pg/ml	250	400	50		100		100	
Std F 2.5 pg/ml	250	400	50		100		100	
Std G 1.25 pg/ml	250	400	50		100		100	
Control NORMAL	250	400	50		100		100	
Control ELEVATED	250	400	50		100		100	**Aspirate supernatant (except T tubes) and count for 1 minute**
Sample	250	400	50		100		100	

APPENDIX IV
SYMBOLS

Symbol	Explanation
	Use By
REF	Catalogue number
LOT	Batch code
	Upper limit of temperature
	Temperature limitation
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Radioactive Material

Symbol	Explanation
BUF H3PO4	Phosphate Buffer
BUF DIL	Antibody Dilution Buffer
Ab	Antiserum
TR	Tracer
CAL	Calibrator
CONTROL N	Control Normal
CONTROL H	Control High
Ab2	2nd Antibody