Explore our innovative technologies for your research

RAT ATRIOPEPTIN

ENZYME IMMUNOASSAY KIT

catalogue # A05103

96 wells

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U.S. patent # 50 47 330
European patent # 89 139 552

THE RAT ATRIOPEPTIN ENZYME IMMUNOASSAY HAS BEEN DEVELOPED AND VALIDATED BY SPI-BIO.

For research laboratory use only.
Not for human diagnostic use.

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RAT ATRIOPEPTIN EIA KIT

96 wells
Storage: -20°C
Expiry date: stated on the package

This kit contains:

- ☞ A covered 96 well plate, pre-coated with mouse anti-rabbit IgG, ready to use after thawing
- ☞ One vial of Rat atriopeptin tracer, lyophilised
- ☞ Two vials of Rat atriopeptin standard, lyophilised
- ☞ One vial of Rat atriopeptin antiserum, lyophilised
- ☞ One vial of EIA buffer, lyophilised
- ☞ One vial of concentrated Wash buffer, liquid
- ☞ One vial of tween 20, liquid
- ☞ Two vials of Quality Control sample, lyophilised
- ☞ Two vials of Ellman's reagent, lyophilised
- ☞ One instruction booklet
- ☞ One template sheet
- ☞ One well cover sheet

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 33 samples in duplicate.

PRECAUTIONS FOR USE

Users are recommended to read all instructions for use before starting work.

Each time a new pipet tip is used, aspirate a sample or reagent and dispense it back into the same vessel. Repeat this operation two or three times before distribution.

For research laboratory use only.
Not for human diagnostic use.
Do not pipet liquids by mouth.
Do not use kit components beyond the expiration date.
Do not eat, drink or smoke in area in which kit reagents are handled.
Avoid splashing.

The total amount of reagents contains less than 100 µg of sodium azide. Flush the drains thoroughly to prevent the production of explosive metal azides.

PRINCIPLE OF THE ASSAY

This Enzyme Immunoassay (EIA) is based on the competition between unlabelled rat atriopeptin and acetylcholinesterase (AChE) linked to rat atriopeptin (tracer) for limited specific rabbit anti-rat atriopeptin antiserum sites.

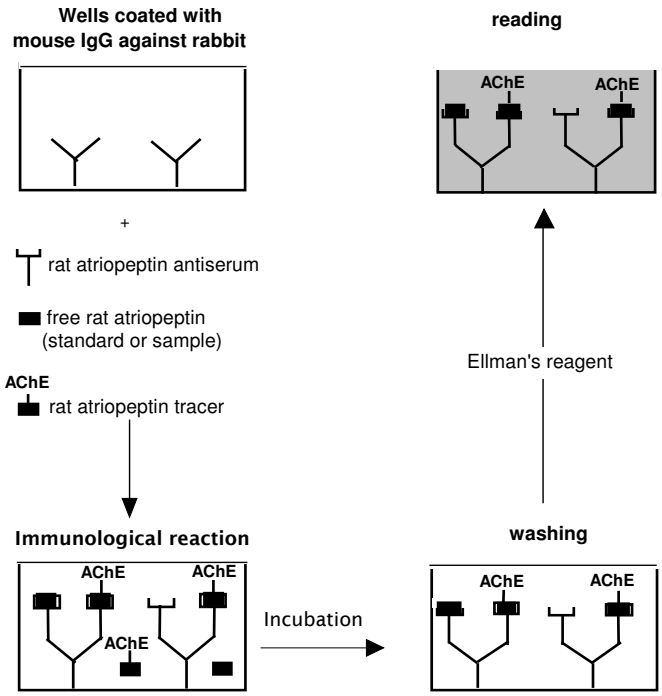
The complex rabbit antiserum-rat atriopeptin (free atriopeptin or tracer) binds to the mouse monoclonal anti-rabbit antibody that is attached to the well.

The plate is then washed and Ellman's Reagent (enzymatic substrate for AChE and chromogen) is added to the wells.

The AChE tracer acts on the Ellman's Reagent to form a yellow compound.

The intensity of the colour, which is determined by spectrophotometry, is proportional to the amount of tracer bound to the well and is inversely proportional to the amount of free rat atriopeptin presents in the well during the immunological incubation.

The principle of the assay is summarised below:



MATERIALS AND EQUIPMENT REQUIRED

In addition to standard laboratory equipment, the following material is required:

FOR SAMPLE PREPARATION

☞ Sodium citrate

☞ EDTA vacutainer tube

☞ C-18 reverse phase cartridges or Oasis® HLB Extraction cartridges

☞ Methanol

☞ Acetic acid

☞ Triethanolamine

☞ Distilled or deionized water

FOR THE ASSAY

☞ Precision micropipettes (20 to 1000 µL)

☞ Spectrophotometer plate reader (405 or 414 nm filter)

☞ Microplate washer (or washbottles)

☞ Microplate shaker

☞ Distilled or deionized water

☞ Polypropylene tubes

SAMPLE COLLECTION & PREPARATION

CARDIA ATRIA FROM RAT

Cardia atria are removed from anaesthetised rats. The frozen atria are pulverised in liquid nitrogen, extracted in 1.0 M acetic acid, heated to 100°C for 10 minutes, homogenised & centrifuged (28,000 x g for 20 minutes). Supernatants are stored at -70°C until assay.

PLASMA SAMPLES

Blood samples are collected in 1/10 volume of 0.11 M sodium citrate (for rat) or into EDTA (3.6 mM) vacutainer tubes (for human) and are centrifuged at 4000 g, 10 minutes at 4°C. Plasma are collected and kept at -70°C until assay.

For relative measurement, no plasma extraction is required. For absolute quantification, plasma should be purified before assay.

EXTRACTION PROTOCOL

- Activate a 1 mL C-18 reverse phase cartridge or an Oasis® HLB Extraction cartridge (SPI-BIO # D30005 or Waters #WAT094226-HLB-3cc) by first passing 6 mL methanol and 5 mL distilled or deionized water.
- Pass two or three mL of sample slowly (about 2mL/minute) through the cartridge.
- Wash the cartridge with 11 mL distilled water followed by 0.5 ml 80% methanol, 4 mM triethanolamine acetate buffer, pH 4.
- Elute atriopeptin with 3 mL (1 mL at a time with 2 minute pauses between each mL) of 80% methanol, 4mM triethanolamine acetate buffer, pH 4.
- Samples are chilled on ice, lyophilised until dry and reconstituted in 300 µL EIA buffer.

RECOVERY AND CALCULATION

To determine the recovery, the sample may be split into two equal aliquots and one spiked with a known amount of rat atriopeptin (approximately equal to the expected amount in the sample).

The recovery will be determined after purification by comparing the concentration of the spiked and unspiked samples.

Either the original concentration of the sample or the recovery factor can be determined by solving the following equations simultaneously:
z = recovery factor
X/a = original concentration of the unspiked sample in a volume known (a)
(X+Y)/b = concentration of the spiked sample (pg/mL) after adding a known amount (Y) in a final volume (b)
The concentration of the unspiked and spiked samples determined by the EIA are respectively equal to (X/a)z & [(X+Y)/b]z.

EXAMPLE
Volume of the unspiked sample: a = 1mL
Final volume of the spiked sample: b = 2mL
Concentration determined by EIA for the unspiked sample: (X/a)z = 8 pg/mL
Concentration determined by EIA for the spiked sample: [(X+Y)/b]z = 16 pg/mL
Quantity of spike: Y = 30 pg in 1mL

$Xz = 8 \Leftrightarrow z=8/X$
$[(X+30)/2]z = 16 \Leftrightarrow [(X+30)]z = 32$
thus,
$[(X+30)]8/X = 32 \Leftrightarrow X+30 = 4X \Leftrightarrow 3X = 30 \Leftrightarrow X = 10$
and
$Xz = 8 \Leftrightarrow z = 0.8$

NOTE
To minimise the calculations, the standard should be concentrated enough so that the addition of the standard does not alter the volume of the sample (a = b) to any great degree (i.e., the assumption is made that the volume is not changed by the addition of the standard).

REAGENT PREPARATION

The coated microtiter plates and reagents are provided ready to use.

☞ EIA buffer
Reconstitute one vial with 50 mL of distilled or deionized water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion. Stability at 4°C: 1 month.

☞ Rat atriopeptin standard
Reconstitute the vial with 1 mL of distilled or deionized water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion. The concentration of the first standard is 2000 pg/mL. Prepare seven propylene tubes (for the seven other standards) and add 500 µL of EIA buffer into each tube. Add 500µL of the first tube (containing the first standard) to the second tube. Continue this procedure for the other tubes. Thus, standard concentrations are: 2000 (S1), 1000 (S2), 500 (S3), 250 (S4), 125 (S5), 62.5 (S6), 31.25 (S7) and 15.6 pg/mL (S8), respectively. Stability at 4°C: 1 day.

☞ Quality Control
Reconstitute one vial with 1 mL of distilled or deionized water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion. Stability at 4°C: 1 day.

☞ Rat atriopeptin-AChE tracer
Reconstitute one vial with 5 mL of distilled or deionized water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion. Stability at 4°C: 1 week.

☞ Rat atriopeptin antiserum
Reconstitute one vial with 5 mL of distilled or deionized water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion. Stability at 4°C: 1 week.

☞ Wash buffer
Dilute 1 mL of the concentrated Wash buffer to 400 mL with distilled or deionized water. Add 200 µL of tween 20 (Use a magnetic stirrer to mix the contents). Stability at 4°C: 1 week.

☞ Ellman's Reagent
Five minutes before use, reconstitute with 50 mL of distilled or deionized water. The tube contents should be thoroughly mixed. Stability at 4°C and in the dark: 1 day.

ASSAY PROCEDURE

It is recommended to perform the assays in duplicate and to follow the instructions hereafter.

PLATE PREPARATION

Prepare the wash buffer as indicated in the reagent preparation section. Open the plate packet and select the sufficient strips for your assay and place the unused strips back in the packet (stored at 4°C). Rinse each well five times with the wash buffer (300 µL/well).

Just before distributing reagents and samples, remove the buffer from the wells by inverting the plate and shaking out the last drops.

DISTRIBUTION OF REAGENTS AND SAMPLES

A plate set-up is suggested on the following page. The contents of each well may be recorded on the sheet provided with the kit.

PIPETTING THE REAGENTS

Note that the first column should be left empty for blanking Ellman's reagent. All samples and reagents must reach room temperature prior to performing the assay. Use different tips to pipet the buffer, standard, sample, tracer, antiserum and other reagents.

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	NSB	S1	S5	*	*	*	*	*	*	*	*
B	B	NSB	S1	S5	*	*	*	*	*	*	*	*
C	B	B0	S2	S6	*	*	*	*	*	*	*	*
D	B	B0	S2	S6	*	*	*	*	*	*	*	*
E	B	B0	S3	S7	*	*	*	*	*	*	*	*
F	B	B0	S3	S7	*	*	*	*	*	*	*	*
G	B	*	S4	S8	*	*	*	*	*	*	*	*
H	B	*	S4	S8	*	*	*	*	*	*	*	*

B : Blank
NSB : Non Specific Binding
Bo : Maximum Binding
S1-S8 : Standards 1-8
* : Samples or Quality controls

- EIA buffer: Dispense 100 µL to Non Specific Binding (NSB) wells and 50 µL to Maximum Binding (Bo) wells.

- Rat atriopeptin standard: Dispense 50 µL of each of the eight standards (S1 to S8) in duplicate to appropriate wells. Start with the lowest concentration standard (S8) and equilibrate the tip in the next higher standard before pipetting.

- Quality control and samples: Dispense 50 µL in duplicate to appropriate wells. Highly concentrated samples may be diluted in EIA buffer.

- Rat atriopeptin AChE tracer: Dispense 50 µL to each well except the Blank (B) wells.

- Rat atriopeptin antiserum: Dispense 50 µL to each well except the Blank (B) wells and the Non Specific Binding (NSB) wells.

INCUBATING THE PLATES

Cover the plate with a plastic film and incubate for 16-20 hours at 4°C.

DEVELOPING AND READING THE PLATE

Reconstitute the wash buffer and Ellman's Reagent as indicated in reagent preparation section. Empty the plate by turning over and shaking. Then, wash each well five times with the wash buffer (300 µL/well). Dispense 200 µL of Ellman's Reagent to the 96 wells. Incubate in the dark (plate covered with an aluminium sheet) at room temperature. Optimal development is obtained using an orbital shaker. The plate should be read between 405 and 414 nm (yellow colour) when the Maximum Binding (Bo) wells reach an absorbance of 0.2-0.8 unit.

	Enzyme Immunoassay Protocol (Volume are in µL)				
	Blank	Non Specific Binding	Maximum Binding	Standard	Sample
Buffer	-	100	50	-	-
Standard	-	-	-	50	-
Sample	-	-	-	-	50
Tracer	-	50	50	50	50
Antiserum	-	-	50	50	50
	Cover the plate, incubate at 4°C for 16-20h				
	Wash the plate 5 times				
Ellman's reagent	200	200	200	200	200
	Incubate the plate with an orbital shaker in the dark at room temperature				
	Read the plate between 405 and 414 nm				

DATA ANALYSIS

Make sure that your plate reader has subtracted the absorbance readings of the blank well (absorbance of Ellman's reagent) from the absorbance readings of the rest of the plate. If not, do it now.

- Calculate the average absorbance for each NSB, Bo, standards and samples.
- Calculate the B/Bo (%) for each standard and sample: (average absorbance of standards or sample - average absorbance of NSB) divided by (average absorbance of Bo - average absorbance of NSB) & multiplied by 100.
- Using a semi-log graph paper, plot the B/Bo (%) for each standard point (y axis) versus the concentration (x axis). Draw a best-fit line through the points.
- To determine the concentration of your samples, find the B/Bo (%) value on the y axis. Read the corresponding value on the x axis which is the concentration of your unknown sample. Samples with a concentration greater than 1000 pg/mL should be re-assayed after dilution in EIA buffer.
- Most plate readers are supplied with curve-fitting software capable of graphing this type of data (logit/log or 4-parameter). If you have this type of software, we recommend using it. Refer to it for further information.

TYPICAL DATA

EXAMPLE DATA

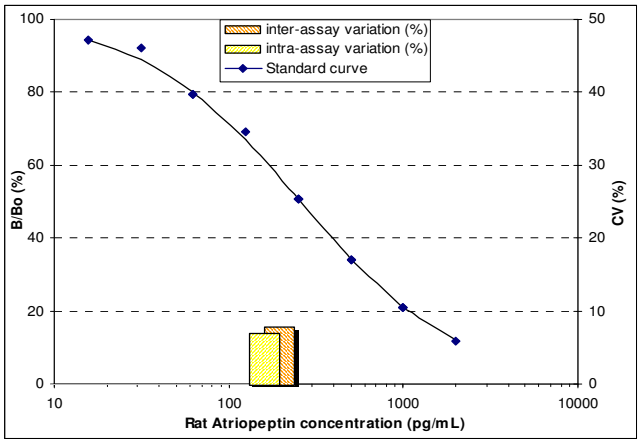
The following data are for demonstration purpose only. Your data may be different and still correct. These data were obtained using all reagents as supplied in this kit under the following conditions: 90 minutes developing at 20°C, reading at 414 nm. A logit/log curve fitting was used to determine the concentrations.

	mAU	B/Bo (%)
NSB	2	-
Bo	392	100
Standard 2000 pg/mL	47	11.7
Standard 1000 pg/mL	84	21.0
Standard 500 pg/mL	134	34.0
Standard 250 pg/mL	199	50.6
Standard 125 pg/mL	271	69.1
Standard 62.5 pg/mL	313	79.5
Standard 31.3 pg/mL	362	92.3
Standard 15.6 pg/mL	370	94.4
QC	201	51.3

ACCEPTABLE RANGE

- Bo absorbance: > 200 mAU in the conditions indicated above.
- Ratio NSB absorbance / Bo absorbance: < 0.1.
- 50% B/Bo (%): 160 to 240 pg/mL (mean: 200 pg/mL).
- QC sample: See the label on the vial.

RAT ATRIOPEPTIN STANDARD CURVE



ASSAY VALIDATION AND CHARACTERISTICS

Assay characteristics are the following:

☞ Cross-reactivity (%):

Rat AP 24	100	ANP (8-33)	100
Rat α-ANP	100	ANP(18-28)	60
Rat ANP (13-28)	1	Auriculin A	10
Rat atriopeptin I	<0.01	BNP	<0.01
Rat atriopeptin II	5	Somatostatin	<0.01
Human ANP	100	Oxytocin	<0.01
Human ß ANP	50	Arg^8-Vasopressin	<0.01
Human γ ANP	40	Urodilatin	100

☞ The limit of detection calculated as the concentration of atriopeptin corresponding to the NSB average minus three standard deviations: 15.6 pg/mL

☞ Standard inter-assay accuracy (n=5):

Atriopeptin standard	Accuracy	Atriopeptin standard	Accuracy
15.6 pg/mL	11.9 %	250 pg/mL	4.6 %
31.2 pg/mL	24.5 %	500 pg/mL	11.6 %
62.5 pg/mL	8.6 %	1000 pg/mL	13.1 %
125 pg/mL	12.5 %	2000 pg/mL	11.6 %

☞ Intra-assay variation & accuracy (n=8) of a 200 pg/mL spiked sample:

	Concentration measured		Repeatability
	(pg/mL)	(%)	(%)
Mean value	202.6	101.3	6.1
S.D.	12.4	-	-
Maximum	220.0	110.0	-
Minimum	178.1	89.1	-

☞ Inter-assay variation & accuracy (n=4) of a 200 pg/mL spiked sample:

	Concentration measured		Reproducibility
	(pg/mL)	(%)	(%)
Mean value	197.4	98.7	6.9
S.D.	13.6	-	-
Maximum	215.7	107.9	-
Minimum	183.1	91.6	-

☞ Limit of quantification: 60 pg/mL

ASSAY TROUBLE SHOOTING

☞ Bo value is too low: incubation in wrong conditions (time or temperature) or reading time too short or Rat atriopeptin-AChE tracer, Rat Atriopeptin antiserum or Ellman's reagent haven't been dispensed.

☞ NSB value too high: contamination of NSB wells with atriopeptin antiserum or inefficient washing.

☞ High dispersion of duplicates: poor pipetting technique or irregular plate washing.

☞ IC_{50} or QC concentrations not within the expected range: wrong preparation of standards.

☞ Analyses of two dilutions of a biological sample do not agree: Interfering substances are present. Sample must be purified prior to EIA analysis (excepting plasma samples).

These are a few examples of trouble shooting that may occur. If you need further explanation, SPI-BIO will be happy to answer any questions or information about this assay. Please feel free to contact our technical support staff by letter, phone (33 (0)1 39 30 62 60), fax (33 (0)1 39 30 62 99) or E-mail (sales@spibio.com), and be sure to indicate the lot number of the kit (see outside of the box).

SPI-BIO offers a training workshop in EIA practice & theory. This workshop is given twice a year. For further information, please contact our Customer Relation Representative (33 (0)1 39 30 62 60).

BIBLIOGRAPHY

☞ **McLaughlin L., Wei Y., Stockmann P., Leahy K., Needleman Ph., Grassi J. & Pradelles Ph.**
Development, validation and application of an enzyme immunoassay of atriopeptin.
Biochem. & Bioph. Res. Co. 144(1), 469-476, 1987

☞ **Grassi J. & Pradelles Ph.**
Compounds labelled by the acetylcholinesterase of *Electrophorus Electricus*. Its preparation process and its use as a tracer or marquer in enzymo-immunological determinations.
United States patent, N°1,047,330. September 10, 1991