Instructions for Use

cyclic AMP RIA

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REF **RIA-5517**

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Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.

Table of Contents

RADIOIMMUNOASSAY FOR THE IN VITRO DETERMINATION OF CYCLIC AMP IN PLASMA AND URINE
For *in vitro* diagnostic use.

1 PRINCIPLE

The radioimmunoassay of cyclic AMP (cAMP) is a competition assay.

Samples or calibrators are incubated in monoclonal antibody coated tubes in presence of 125I-labeled cAMP tracer. Following incubation, the contents of the tubes are aspirated and bound radioactivity is measured in a gamma counter. A calibration curve is established and unknown values are determined by interpolation from the standard curve.

2 WARNING AND PRECAUTIONS

2.1 General remarks

- The vials with calibrators should be opened as shortly as possible to avoid excessive evaporation.
- Bring all reagents to room temperature before pipeting.
- Do not mix the reagents from kits of different lots.
- A standard curve must be included with each assay.
- It is recommended to perform the assay in duplicate.
- Each tube must be used only once.

2.2 Basic rules of radiation safety

The purchase, possession, utilization, and transfer of radioactive material is subject to the regulations of the country of use. Adherence to the basic rules of radiation safety should provide adequate protection:

- No eating, drinking, smoking or application of cosmetics should be carried out in the presence of radioactive materials.
- No pipeting of radioactive solutions by mouth.
- Avoid all contact with radioactive materials by using gloves and laboratory overalls.
- All manipulation of radioactive substances should be done in an appropriate place, distant from corridors and other busy places.
- Radioactive materials should be stored in the container provided in a designated area.
- A record of receipt and storage of all radioactive products should be kept up to date.
- Laboratory equipment and glassware which are subject to contamination should be segregated to prevent cross-contamination of different radioisotopes.
- Each case of radioactive contamination or loss of radioactive material should be resolved according to established procedures.
- Radioactive waste should be handled according to the rules established in the country of use.

2.3 Sodium azide

Some reagents contain sodium azide as a preservative. Sodium azide can react with lead, copper or brass to form explosive metal azides. Dispose of the reagents by flushing with large amounts of water through the plumbing system.

2.4 Material of human origin

All plasma samples should be handled as if capable of transmitting hepatitis or AIDS and waste should be discarded according to the country rules.

2.5 GHS Hazard Classification

Not classified as hazardous

The Safety Data Sheet (SDS) is available upon request.

3 SPECIMEN COLLECTION, PROCESSING, STORAGE AND DILUTION

The procedures for sampling should be rigorously observed in order to prevent the degradation of cAMP. If the samples have been frozen at <-18 °C, thaw at 18 °C - 25 °C until a complete thawing and mix carefully before the assay.

Waste should be discarded according to the country rules.

Plasma

Collect blood in tubes containing EDTA and cool immediately on ice.

Separate plasma from cells by centrifugation at 4 °C, within 20 minutes following sampling.

Separate plasma and freeze at <-18 °C if the assay cannot be done immediately.

Urine

Collect a 24-hours urine specimen in tubes containing a preservative.

Urine may be stored at 2 °C - 8 °C if cAMP is measured within 24 hours.

For longer storage, freeze at <-18 °C.

4 MATERIALS PROVIDED

All reagents of the kit are stable until the expiry date indicated on the kit labels, if stored at 2 °C - 8 °C.

Note: Temperatures and expiry dates printed on vial labels apply to the long-term storage of components by the manufacturer only, prior to assembly of the kit. Do not take into account.

Anti-cAMP antibody-coated tubes: 2 x 50 tubes (ready-to-use)

^{125}I-labeled cAMP tracer: one vial (lyophilized)

The vial contains 111 kBq at time of manufacture of ^{125}I-labeled cAMP, lyophilized with bovine serum albumin.

Reconstitute the content of the vial with 55 mL of diluent.
After reconstitution store the tracer at 2 °C - 8 °C for no longer than 48 hours.
For longer periods, store the vial at <-18 °C until the expiration date of the kit.

Calibrators: six vials (lyophilized)

The calibrator vials contain from 0 to approximately 50 nM of cAMP, lyophilized with bovine serum albumin.

Reconstitute the content of each vial with 1 mL of diluent.
The exact concentrations are indicated on each vials label.
After reconstitution, store the calibrators at 2 °C - 8 °C for no longer than 24 hours.
For longer periods, store the vials at <-18 °C until the expiration date of the kit.

Calibrators are verified to an internal reference standard.

Diluent (10 X): one 50 mL concentrated solution vial

The 50 mL of concentrated solution have to be diluted with 450 mL of distilled water before use.

The vial contains sodium azide (<0.1%).

The diluent is stable at 2 °C - 8 °C until the expiration date of the kit.

5 MATERIALS REQUIRED, BUT NOT PROVIDED

In addition to standard laboratory equipment, the following items are required:

- precision micropipets (50 µL,100 µL).
- repeating micropipet (500 µL).
- "vortex" type mixer.
- aspiration system.
- gamma counter.

6 RESULTS

Results are obtained from the standard curve by interpolation. The curve serves for the determination of cAMP concentrations in samples measured at the same time as the calibrators.

Standard curve

The results in the quality control department were calculated using *spline* curve fit with B/T or B/B_0 on the logit vertical axis and the analyte concentration of the calibrators on the log horizontal axis (nM).

Other data reduction methods may give slightly different results.

Total activity: 32,105 cpm				
Calibrators	**cAMP (nM)**	**cpm (n=3)**	**B/T (%)**	**B/B_0 (%)**
0	0	23,841	74.3	100
1	0.52	17,697	55.1	75.4
2	1.48	12,263	38.2	52.2
3	5.40	8,631	20.7	28.2
4	15.8	3,413	10.6	14.5
5	54.0	1.925	6.0	8.2

(Example of standard curve, do not use for calculation)

Samples

Locate the B/T or the B/B_0 for each sample on the vertical axis.

Correct for initial dilution of samples and read off corresponding cAMP concentration in nM on the horizontal axis.

Nephrogenic cAMP (cAMPn)

Output dn of nephrogenic cAMP in nM/minute:

$$dn = [(Cu \times d) - (Cp \times Cl)] \times 10^{-3}$$

Cu = urinary cAMP in nM

Cp = plasma cAMP in nM

d = output of urine in mL/minute

Cl = clearance of creatinine in mL/minute

Concentration Cn of nephrogenic cAMP in the urine in nM of glomerular filtrate:

$$Cn = (Cu \times Crp / Cru) - Cp$$

Crp = plasma creatinine

Cru = urinary creatinine

7 EXPECTED VALUES

We recommend each laboratory to establish its own reference values. The following values obtained from healthy subjects are indicative only.

Number of subjects	**Min. - Max.**	**2.5th - 97.5th percentile**
50	15.95 – 47.63 nM	17.97 – 45.40 nM

	N	**Min.**	**Max.**	**Median**	**2.5th percentile**	**97.5th percentile**
		µmol				
Urine (24 hour)	30	1.23	10.60	5.67	2.26	10.00

8 QUALITY CONTROL

Good laboratory practices imply that control samples be used regularly to ensure the quality of the results obtained. These samples must be processed exactly the same way as the assay samples, and it is recommended that their results be analyzed using appropriate statistical methods.

In case of packaging deterioration or if data obtained show some performance alteration, please contact your local distributor or DRG.

9 PROCEDURE

9.1 Preparation of reagents

- Let the reagents come to room temperature.
- Dilute the 50 mL of diluent concentrated solution with 450 mL of distilled water.
- Reconstitute the content of each vial of calibrator with 1 mL of diluent.
- Reconstitute the tracer with 55 mL of diluent.

9.2 Preparation of samples

- Dilute plasma 1:11 in diluent (50 µL of plasma + 500 µL of diluent).
- Dilute urine 1:1000 in diluent.

9.3 Assay procedure

Immunological step	Washing step	Counting
To antibody coated tubes add 100 µL of sample, or calibrator and 500 µL of tracer* vortex gently Incubate 18 hours at 2 °C - 8 °C	Aspirate carefully the content of each tube while still cold (except "total cpm").	Count for 1 minute bound cpm (B) and total cpm (T).

*Add 500 µL of tracer to 2 additional tubes to obtain total cpm.

10 PERFORMANCE CHARACTERISTICS

(For more details, see the data sheet "APPENDIX")

10.1 Sensitivity

Analytical sensitivity: 0.06 nM

Functional sensitivity: 0.08 nM

10.2 Specificity

The kit is specific of the cAMP.

10.3 Precision

10.3.1 Intra-assay

Samples were assayed 25 times in the same series. The coefficients of variation were found below or equal to 10.3 % for plasma and below or equal to 10.6 % for urine.

10.3.2 Inter-assay

Samples were assayed in duplicate in 10 different series. Coefficients of variation were found below or equal to 9.0 % for plasma and below or equal to 10.2 % for urine.

10.4 Accuracy

10.4.1 Dilution test

High-concentration samples were serially diluted in diluent. The recovery percentages obtained were between 90.7 % and 118 % for plasma and between 87.6 % and 116 % for urine.

10.4.2 Recovery test

Low-concentration samples were spiked with known quantities of cAMP. The recovery percentages obtained were between 85.9 % and 110 % for plasma and between 92.6 % and 102 % for urine.

10.5 Measurement range

(from analytical sensitivity to highest calibrator): 0.06 to approximately 50 nM.

11 LIMITATIONS

The non-respect of the instructions in this package insert may affect results significantly.

Results should be interpreted in the light of the total clinical presentation of the patient, including clinical history, data from additional tests and other appropriate information.

For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays.

Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

11.1 Interference

Results obtained with icteric and hemolysed samples must be interpreted with caution.

The measurement of cAMP at very low concentrations in presence of very high quantities of ATP must be carried out according to Volker T.T., Viratelle O.M., Delaage M.A. & Labouesse J. (1985), Anal. Biochem., 144, 347-355.

Heparin does not interfere with the assay but does not protect cAMP against enzymatic degradation therefore samples must be kept in cold.

It is possible to measure cAMP in the serum with the same protocol as for plasma, but because the phosphodiesterase activity in the serum, the serum value is always lower than the plasma value.

12 APPENDIX - PERFORMANCE CHARACTERISTICS

12.1 Specificity

Data on cross-reactivity with several related molecules are presented in the following table.

Cross-reactivity (%) = (cAMP / related molecule) x 100 at 50 % of the binding of the zero calibrator.

Related molecule	Cross-reactivities (%)
cAMP	100
cGMP	0.006
5'AMP	0.004
ATP	0.02

12.2 Precision

12.2.1 Intra-assay

EDTA plasma samples	P1	P2	P3
Number of determinations	25	25	25
Mean value, nmol/L	0.94	3.55	7.20
C.V., %	10.26	6.53	3.46

Urine samples	U1	U2	U3
Number of determinations	25	25	25
Mean value, nmol/L	1.02	5.17	38.99
C.V., %	10.64	4.68	6.17

12.2.2 Inter-assays

EDTA plasma samples	P1	P2	P3
Number of determinations	10	10	10
Mean value,nmol/L	1.19	5.99	27.67
C.V., %	9.03	4.37	6.56

Urine samples	U1	U2	U3
Number of determinations	10	10	10
Mean value, nmol/L	0.85	4.80	47.95
C.V., %	10.17	4.69	4.69

12.3 Accuracy

12.3.1 Dilution test

Three samples were diluted in diluent and assayed according to the assay procedure.

EDTA plasma samples	Dilution factor	cAMP (nmol/L)		Ratio (%) Measured/ Expected
		Measured	Expected	
P1	1:10	18.82	-	-
	1:20	11.14	9.41	118
	1:40	5.08	4.71	108
	1:80	2.59	2.35	110
	1:160	1.29	1.18	110
	1:320	0.55	0.59	93.5
P2	1:10	>STD	-	-
	1:20	18.73	-	-
	1:40	8.59	9.37	91.7
	1:80	4.57	4.68	97.6
	1:160	2.39	2.34	102
	1:320	1.13	1.17	96.5
P3	1:10	>STD	-	-
	1:20	24.76	-	-
	1:40	11.23	12.38	90.7
	1:80	5.64	6.19	91.1
	1:160	2.81	3.10	90.8
	1:320	1.53	1.55	98.9

Urine sample	Dilution factor	cAMP (nmol/L)		Ratio (%) Measured/ Expected
		Measured	Expected	
U1	1:100	47.44	-	-
	1:200	26.24	23.72	111
	1:400	13.72	11.86	116
	1:800	6.29	5.93	106
	1:1600	3.00	2.97	101
	1:3200	1.51	1.48	102
U2	1:100	51.85	-	-
	1:200	23.21	25.93	89.5
	1:400	11.56	12.96	89.2
	1:800	6.02	6.48	92.9
	1:1600	2.96	3.24	91.3
	1:3200	1.42	1.62	87.6
U3	1:125	43.88	-	-
	1:250	19.77	21.94	90.1
	1:500	10.23	9.89	103
	1:1000	4.86	4.94	98.3
	1:2000	2.35	2.47	95.1
	1:4000	1.10	1.24	89.0

12.4 Recovery test

To sample with low concentration of cAMP were added known concentrations of cAMP. Samples were assayed according to the kit procedure.

EDTA plasma samples	Endogen. conc. (nmol/L)	Added conc. (nmol/L)	Expected conc. (nmol/L)	Measured conc. (nmol/L)	Ratio (%) Measured/ Expected
P1	0.28	0.29	0.57	0.49	85.9
	0.28	0.62	0.89	0.80	89.6
	0.27	1.23	1.50	1.35	90.3
P2	0.62	0.77	1.39	1.35	97.1
	0.63	1.55	2.18	2.18	100
	0.61	3.01	3.62	3.71	103
P3	1.20	1.17	2.37	2.61	110
	1.17	2.29	3.46	3.68	106
	1.12	4.55	5.66	5.72	101

Urine Samples	Endogen. conc. (nmol/L)	Added conc. (nmol/L)	Expected conc. (nmol/L)	Measured conc. (nmol/L)	Ratio (%) Measured/ Expected
U1	0.64	0.29	0.92	0.93	101
	0.62	0.62	1.24	1.19	96.1
	0.60	1.23	1.83	1.69	92.6
U2	1.53	0.77	2.31	2.27	98.3
	1.57	1.55	3.12	2.89	92.6
	1.52	3.01	4.53	4.35	96.0
U3	1.94	1.17	3.12	3.18	102
	1.90	2.29	4.19	4.28	102
	1.81	4.55	6.35	5.94	93.5

12.5 ^{125}I Characteristics

$T_{1/2}$ (^{125}I) = 1443 h = 60.14 d

^{125}I	E (MeV)	%
γ	0.035	
X	0.027	114
	0.032	25

SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Dispositivo medico-diagnostico in vitro	Producto sanitario para diagnóstico In vitro	Dispositif médical de diagnostic in vitro
REF	Catalogue number *	Artikelnummer *	Numero di Catalogo	Nûmero de catálogo	Référence de catalogue
LOT	Batch code *	Chargencode *	Codice del lotto	Codigo de lote	Numéro de lot
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use-by date *	Verwendbar bis *	Utilizzare prima del	Establa hasta	Utiliser jusque
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
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