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**Instructions for use**

# Dopamine Plasma RIA High Sensitive

**REF** **BA R-4300**

96

+2 +8 °C

IVD

CE

200 kBq

## 1. Principle of the test

High-sensitive Radioimmunoassay for the quantitative determination of dopamine in plasma

Dopamine is extracted from a plasma sample*) by using a cis-diol-specific affinity gel, acylated and then modified enzymatically.
The assay procedure follows the basic principle of radioimmunoassay, involving competition between a radioactive and a non-radioactive antigen for a fixed number of antibody binding sites. The amount of 125I-labelled antigen bound to the antibody is inversely proportional to the analyte concentration of the sample. After the system is in equilibrium, the antibody bound radioactivity is precipitated with a second antibody in the presence of polyethylene glycol. The precipitate is counted in a gamma counter. Quantification of unknown samples is achieved by comparing their activity with a reference curve prepared with known standards.

*) Flexible sample volumes between 100 – 600 µl can be used with this assay.

## 2. Advice on handling the test

### 2.1 Reliability of the test results

In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, RILIBÄK, etc.). Special attention must be paid to control checks for precision and correctness during the test; the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions.
It is recommended that each laboratory establishes its own reference intervals. The values reported in this test instruction are only indicative.
The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.

### 2.2 Complaints

In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

### 2.3 Warranty

This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

### 2.4 Disposal

Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.
The appropriate safety data sheets of the individual products are available on the homepage. The safety data sheets correspond to the standard: ISO 11014-1.

### 2.5 Interference

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

### 2.6 Precautions

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation.

The radioactive material may be received, acquired, possessed, and used only by physicians, veterinarians in the practice of veterinary medicine, clinical laboratories or hospitals and only for in vitro clinical or laboratory tests not involving internal or external administration of the material, or the radiation there from, to human beings or animals. Its receipt, acquisition, possession, use, and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority. In no case the product must be administered to humans or animals.
All radioactive handling should be executed in a designated area, away from regular passage. A log book for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.
Any radioactive spills must be cleaned immediately in accordance with the radio safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.

## 3. Storage and stability

The reagents should be stored at 2 - 8 °C. Do not use components beyond the expiration date shown on the kit labels.

### 4.1 Contents of the kit

| | | | | |
|---|---|---|---|---|
| **BA R-0030** | PREC-REAG | **Precipitating Reagent** | 1x 55 mL | ready for use, goat anti-rabbit serum in PEG phosphate buffer. *Mix thoroughly before use!* |
| **BA R-0050** | ADJUST-BUFF | **Adjustment Buffer** | 1 x 4 mL | ready for use |
| **BA R-0320** | $^{125}$I DOP | **$^{125}$I – Dopamine** | 1 x 5.5 mL | activity < 200 kBq, ready for use, red coloured, green screw cap |
| **BA R-4310** | AS DOP | **Dopamine Antiserum** | 1 x 5.25 mL | from rabbit, ready for use, green coloured, green screw cap |
| **BA R-4601** | STANDARD A | **Standard A** | 1 x 4 mL | ready for use |
| **BA R-4602** | STANDARD B | **Standard B** | 1 x 4 mL | ready for use |
| **BA R-4603** | STANDARD C | **Standard C** | 1 x 4 mL | ready for use |
| **BA R-4604** | STANDARD D | **Standard D** | 1 x 4 mL | ready for use |
| **BA R-4605** | STANDARD E | **Standard E** | 1 x 4 mL | ready for use |
| **BA R-4606** | STANDARD F | **Standard F** | 1 x 4 mL | ready for use |
| **BA R-4617** | TE-BUFF | **TE Buffer** | 1 x 4 mL | ready for use |
| **BA R-4651** | CONTROL 1 | **Control 1** | 1 x 4 mL | ready for use |
| **BA R-4652** | CONTROL 2 | **Control 2** | 1 x 4 mL | ready for use |
| **BA R-6611** | ACYL-BUFF | **Acylation Buffer** | 1 x 20 mL | ready for use |
| **BA R-6612** | ACYL-REAG | **Acylation Reagent** | 1 x 3 ml | ready for use |
| **BA R-6614** | COENZYME | **Coenzyme** | 1 x 4 mL | ready for use, S-adenosyl-L-methionine |
| **BA R-6615** | ENZYME | **Enzyme** | 2 x 1 mL | lyophilized, contains the enzyme catechol-O-methyltransferase |
| **BA R-6618** | EXTRACT-PLATE 48 | **Extraction Plate** | 2 x 48 wells | coated with boronate affinity gel |
| **BA R-6619** | HCL | **Hydrochloric Acid** | 1 x 20 mL | ready for use, yellow coloured, contains 0.025 M HCl |

## 4.2 Additional materials and equipment required but not provided with the kit

- Calibrated variable precision micropipettes (e.g. 1-10 µL / 10-100 µL / 100-1000 µL)
- Polystyrene tubes and suitable rack
- Temperature controlled water bath, heating block or incubator (37 °C)
- Centrifuge capable of at least 3,000 x g
- Suitable device for aspirating or decanting
- Plate shaker (shaking amplitude 3mm; approx. 600 rpm)
- Gamma counter
- Vortex mixer
- Distilled water

## 5. Sample collection and storage

**Plasma**

EDTA-Plasma should be used. Do not use haemolytic or lipemic samples.
Storage: up to 6 hours at 2 - 8°C; for longer periods (up to 6 months) at - 20°C.
Repeated freezing and thawing should be avoided.

## 6. Test procedure

A plasma volume between **100 µl-600 µl** is needed per single determination.

If a plasma volume **< 600 µl** is used, dist. water has to be added to a **final volume of 600 µl** and this **prediluted sample** has to be used for the extraction procedure (please refer to point 6.2 of this protocol).

This sample predilution has to be considered in the calculation of results (please refer to point 7 of this protocol).

### 6.1 Preparation of reagents

**Enzyme Solution**

Reconstitute the content of the vial labelled 'Enzyme' with 1 mL distilled water and mix thoroughly. Add 0.3 mL of Coenzyme followed by 0.7 mL of Adjustment Buffer. The total volume of the enzyme solution is 2.0 mL.

⚠ *The Enzyme Solution has to be prepared freshly prior to the assay (not longer than 10 - 15 minutes in advance). Discard after use!*

Allow all reagents – with the exception of the Precipitating Reagent - to reach room temperature and mix thoroughly by gentle inversion before use. Number the assay tubes accordingly. Duplicate determinations are recommended.

⚠ *Pipetted liquids should not adhere to the wall of the RIA tubes. If necessary please centrifuge the tubes for 1 minute at 500xg to spin down adhering liquids.*

### 6.2 Sample preparation, extraction and acylation

| | |
|---|---|
| **1.** | Pipette **30 µL** of **standards**, **controls** and **600 µL** of **plasma samples** (diluted or undiluted) into the respective wells of the **Extraction Plate**. |
| **2.** | Add **500 µL** of **distilled water** to the wells with **standards** and **controls.** |
| **3.** | Pipette **25 µL** of **TE Buffer** into all wells |
| **4.** | Cover the plate with adhesive foil. Shake **60 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm). |
| **5.** | Remove the foil and empty the plate. Blot dry by tapping the inverted plate on absorbent material. |
| **6.** | Pipette **1 mL** of **distilled water** into all wells. |
| **7.** | Shake **5 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm). |
| **8.** | Blot dry by tapping the inverted plate on absorbent material. |
| **9.** | **Wash one more time** as described (step 6, 7 and 8)! |
| **10.** | Pipette **150 µL** of **Acylation Buffer** into all wells. |
| **11.** | Pipette **25 µL** of **Acylation Reagent** into all wells. |
| **12.** | Shake **20 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm). |
| **13.** | Empty the plate and blot dry by tapping the inverted plate on absorbent material. |
| **14.** | Pipette **1 mL** of **distilled water** into all wells. |
| **15.** | Shake **5 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm). |
| **16.** | Blot dry by tapping the inverted plate on absorbent material. |
| **17.** | **Wash one more time** as described (step 14, 15, 16). |
| **18.** | Pipette **200 µL** of **Hydrochloric Acid** into all wells. |
| **19.** | Cover the plate with adhesive foil. Shake **10 min** at **RT** (20-25°C) on an orbital shaker (approx. 600 rpm). |
| ⚠ | ***Do not decant the supernatant thereafter!*** |
| | The following volumes of the supernatants are needed for the subsequent RIA: |
| | **Dopamine 80 µL** |

### 6.3 Dopamine RIA

| | |
|---|---|
| **1.** | Pipette **80 µL** of **Hydrochloric Acid** into the tubes for the NSB. |
| **2.** | Pipette **80 µL** of **the extracted standards, controls and samples** into the respective tubes. |
| **3.** | Pipette **25 µL** of **Enzyme Solution** (refer to 6.1*)* into all tubes (**except totals**). |
| **4.** | Mix thoroughly and incubate for **2 hours** at **37 °C**. |
| **5.** | Pipette **50 µL** of the corresponding **$^{125}$I Tracer Dopamine** into the respective **tubes**. |
| **6.** | Pipette **50 µL** of the corresponding **Dopamine Antiserum** into the respective **tubes** (***except totals and NSB***); mix thoroughly. |
| **7.** | Cover the tubes. Incubate for **15 - 20 hours** (overnight) at **2 - 8 °C** |
| **8.** | Mix the chilled (2 - 8 °C) **Precipitating Reagent** thoroughly, pipette each **0.5 mL** into all tubes (***except totals***), and mix on a vortex. |
| **9.** | Incubate for **15 minutes** at **2 - 8 °C**. |
| **10.** | Centrifuge for **15 minutes** at **3,000 x g**, if possible in a refrigerated centrifuge. |
| **11.** | **Decant** or aspirate the **supernatant** carefully (***except totals***). Blot the tubes dry and leave them upside for 2 minutes. |
| **12.** | **Count** all tubes for **1 minute** in a gamma counter. |

## 7. Calculation of results

**The standards refer to:**

| | Concentration of the standards | | | | | |
|---|---|---|---|---|---|---|
| **Standard** | **A** | **B** | **C** | **D** | **E** | **F** |
| **Dopamine (pg/mL)** | 0 | 20 | 60 | 200 | 800 | 3 200 |
| **Dopamine (pmol/L)** | 0 | 131 | 392 | 1 306 | 5 224 | 20 896 |
| **Conversion:** | Dopamine (ng/mL) x 6.53 = Dopamine (nmol/L) | | | | | |

Subtract the mean cpm of the non-specific binding NSB from the mean cpm of standards, controls and samples.
The calibration curve from which the concentrations in the samples can be read off, is obtained by plotting the percentage of (B-NSB)/(B0-NSB) measured for the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).
Use a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

The concentrations of the **undiluted plasma samples** and the **controls** can be read directly from the standard curve.

**Concentration of diluted plasma samples:**
If only a plasma volume **< 600 µl** was used for the extraction, the concentration read from the standard curve has to be **multiplied** with a **volume-factor**:

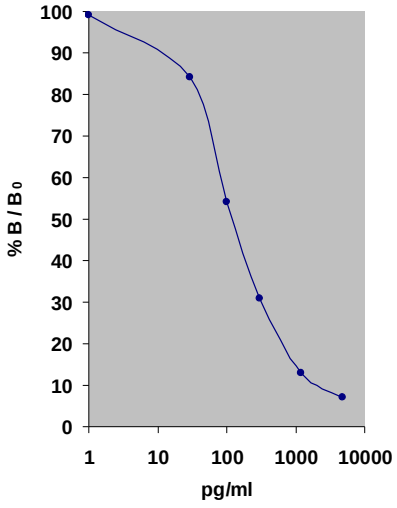
$$\textbf{Volume-factor} = \frac{\textbf{600 µl}}{\textbf{used plasma volume (µl)}}$$

### 7.1 Quality control

It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC-report.

### 7.2 Typical calibration curves

**DOPAMINE**




⚠ *Example. Do not use for calculation!*

## 8. Assay characteristics

| | | |
|---|---|---|
| **Expected Reference Values** | | **Dopamine** |
| | Plasma | < 100 pg/mL |

| | | |
|---|---|---|
| **Analytical Sensitivity** (600 µl undiluted plasma) | | Mean signal (Zero-Standard) - 2SD |
| | | **Dopamine** |
| | Plasma | 7 pg/mL |

| | | |
|---|---|---|
| **Functional Sensitivity** | | Mean concentration < 20% CV |
| | | **Dopamine** |
| | Plasma | 20 pg/mL |

| | **Substance** | **Cross Reactivity (%)** |
|---|---|---|
| | | Dopamine |
| **Analytical Specificity (Cross Reactivity)** | Derivatized Adrenaline | 0.03 |
| | Derivatized Noradrenaline | 0.87 |
| | Derivatized Dopamine | 100 |
| | Metanephrine | < 0.007 |
| | Normetanephrine | 0.008 |
| | 3-Methoxytyramine | 0.55 |
| | 3-Methoxy-4-hydroxyphenylglycol | < 0.007 |
| | Tyramine | 0.13 |
| | Phenylalanine, Caffeinic acid, L-Dopa, Homovanillic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid | < 0.007 |

**Precision**

**Intra-Assay (human EDTA-plasma)**

| | **Sample** | **Mean ± 3 SD (pg/mL)** | **SD (pg/mL)** | **CV (%)** |
|---|---|---|---|---|
| Dopamine | high | 1438.6 ± 465.6 | 155.2 | 10.8 |
| | medium | 565.9 ± 246.3 | 82.1 | 14.5 |
| | low | 56.4 ± 36.3 | 12.1 | 21.5 |

| **Recovery** (human EDTA-plasma) | **Mean (%)** | **Range (%)** | **SD (%)** | **CV (%)** |
|---|---|---|---|---|
| Dopamine | 97.7 | 83.7 – 115.9 | 11.8 | 12.1 |

⚠ ***For updated literature, information about clinical significance or any other information please contact your local supplier.***

**Symbols:**

| | | | | | |
|---|---|---|---|---|---|
| +2 +8 °C | Storage temperature | | Manufacturer | Σ | Contains sufficient for <n> tests |
| | Expiry date | LOT | Batch code | IVD | For in-vitro diagnostic use only! |
| | Consult instructions for use | CONT | Content | CE | CE labelled |
| | Caution | REF | Catalogue number | RUO | For research use only! |