

Manufactured for:

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Instructions for use

Melatonin (Research) RIA

¹²⁵I – Radioimmunoassay reagents for the direct determination of Melatonin in various biological types.

For Research Use Only, Not for Use in Diagnostic Procedures.

REF

IB88139



RUO

For Research use only-
Not for use in diagnostic
procedures

< 200 KBq
< 5.40 µCi

Melatonin Research RIA

1. **Intended use**

¹²⁵I – Radioimmunoassay reagents for the direct determination of Melatonin in various biological types. For research use only, not for use in diagnostic procedures.

2. **Principle of the test**

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 unknown being tested. When 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. Determination of unknowns is achieved by comparing their activity with a reference curve prepared with known calibrators.

3. **Procedural Notes**

3.1 **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.

3.2 **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.

3.3 **Interference**

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of the biological being tested 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.

3.4 **Precautions**

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and biologicals with skin. No smoking, eating or drinking in areas where biologicals or kit test tubes are handled. When working with kit components or unknowns, always wear protective gloves and wash your hands 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.

This kit contains ¹²⁵Iodine (half life: 60 days), emitting ionizing X- (28 kev) and G- (35.5 kev) radiations. The receipt, acquisition, possession, use, and transfer of radioactive materials are subject to license and regulations of the U.S. Nuclear Regulatory Commission or the State with which the NRC has entered into an agreement for the exercise of regulatory authority. Radioactive material may be received, acquired, possessed, and used only by qualified authorized personnel and only for laboratory tests not involving internal or external administration of the material, or the radiation there from, to human beings or animals.

All radioactive material should be stored in specially designated areas. Handling and use of radioactive materials should be done only in designated areas by authorized personnel. Any radioactive spills must be cleaned immediately and all contaminated materials disposed of as radioactive waste in accordance with the appropriate regulatory authority and radio safety procedures. Adherence to the basic rules of laboratory and radiation safety provides adequate protection.

All reagents of this test kit which contain human or animal serum or plasma have been tested and confirmed negative for HIV I/II, HbsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.

4. **Storage and stability**

The reagents should be stored at 2 - 8 °C until expiration date. Do not use components beyond the expiration date shown on the kit labels. Do not mix various lots of any kit component within an individual assay. Melatonin is sensitive to light-exposure. To avoid photo-oxidative reduction of melatonin, it is necessary to keep it away from direct sun light and from heat.

5.1 **Contents of the kit**

BA R-3301	CALIBRATOR A	Calibrator A	1 x 4 mL	ready for use
BA R-3302	CALIBRATOR B	Calibrator B	1 x 4 mL	ready for use
BA R-3303	CALIBRATOR C	Calibrator C	1 x 4 mL	ready for use
BA R-3304	CALIBRATOR D	Calibrator D	1 x 4 mL	ready for use
BA R-3305	CALIBRATOR E	Calibrator E	1 x 4 mL	ready for use
BA R-3306	CALIBRATOR F	Calibrator F	1 x 4 mL	ready for use
BA R-3307	CALIBRATOR G	Calibrator G	1 x 4 mL	ready for use
BA R-3310	AS MEL	Melatonin Antiserum	1 x 5.25 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA R-3313	ASSAY-BUFF	Assay Buffer	1 x 15 mL	ready for use
BA R-3315	ENZYME	Enzyme	3 x 3 mL	lyophilized
BA R-3316	ENZYME-BUFF	Enzyme Buffer	1 x 15 mL	ready for use
BA R-3320	¹²⁵ I-MEL	¹²⁵I – Melatonin	1 x 5.5 mL	activity < 200 kBq, ready for use, red coloured, red screw cap
BA R-0030	PREC-REAG	Precipitating Reagent	2 x 55 mL	ready for use, goat anti-rabbit serum in PEG phosphate buffer, yellow screw cap. <i>Mix thoroughly before use!</i>
BA R-3351	CONTROL 1	Control 1	1 x 4 mL	ready for use
BA R-3352	CONTROL 2	Control 2	1 x 4 mL	ready for use
BA R-3961	SYRINGE	Syringe	1 x 3 pcs.	single-use, ready for use
BA R-3962	FILTER UNITS	Filter Units	1 x 3 pcs.	single-use, ready for use
BA R-3963	PREP TUBES	Preparation Tubes	1 x 3 pcs.	ready for use
BA R-3964	CHARCOAL	Charcoal Suspension	1 x 25 mL	ready for use

Calibrator	Concentration of the Calibrators:						
	A	B	C	D	E	F	G
Melatonin (pg/mL)	0	30	100	300	1 000	3 000	10 000
Melatonin (pmol/L)	0	129	430	1 290	4 300	12 900	43 000
Conversion:	Melatonin (pg/mL) x 4.30 = Melatonin (pmol/L)						

5.2 **Additional materials and equipment required but not provided in the kit**

- Calibrated variable precision micropipettes (e.g. 1-10 µL / 10-100 µL / 100-1000 µL)
- Conical plastic tubes (polypropylene, polystyrene) and suitable rack
- Centrifuge (preferable refrigerated) capable of at least 3 000 x g
- Suitable device for aspirating or decanting the tubes
- Vortex mixer
- Gamma counter
- Distilled water

6. **Collection and Handling of Unknowns**

The unknowns have to be stored deep frozen until use.

7. **Test procedure**

Allow all reagents – with the exception of 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.*

⚠ *The use of conical tubes is highly recommended for the assay.*

7.1 **Preparation of reagents**

Enzyme

Reconstitute the content of the vial with 3 mL of Enzyme Buffer prior to use. Mix carefully (30 minutes on a rotating mixer). The reconstituted Enzyme cannot be stored and can only be used once. Upon request additional Enzyme vials are provided.

Equalizing Reagent:

The needed amount of Equalizing Reagent depends on how much volume of unknowns is used for the experiment and has a strong influence on the limit of detection. The following examples demonstrate the relation between number of runs, volume of unknowns and needed Equalizing Reagent:

Number of runs (calibration curves)	Volume of unknowns (µL)	Tubes (N)*	Volume of Equalizing Reagent needed**
1	100	18	1.8 mL
2	100	36	3.6 mL
2	50	36	1.8 mL
2	200	36	7.2 mL
2	400	36	14.2 mL

* The number of tubes needed to prepare a calibration curve in duplicates including NSB (only tracer), 7 calibrators and 2 controls.

** The charcoal treatment of biological fluids in combination with the ultra filtration step always leads to a loss of liquid. Therefore it is recommended to take more volume of unknowns to prepare the Equalizing reagent than is needed in the assay (e.g. twice as much).

Always check the volume of the melatonin free Equalizing Reagent prior to its use in the RIA.

7.2 **100 µL version for 2 assay runs (see 100 µL volume above)**

1.	Pipette 7.2 mL of Charcoal Suspension into the Preparation Tubes .
2.	Spin down the charcoal
3.	Decant the supernatant and remove carefully remaining liquid from the wall of the tubes
4.	Pipette 7.2 mL of the biological fluid (serum, tissue extract etc.) onto the charcoal pellet
5.	Close the tube carefully and mix the suspension for 30 minutes at RT (20-25°C) on a roller mixer .
6.	Spin down the charcoal
7.	Remove charcoal fines by filtering the supernatant. One Filter Unit is capable of filtering 4 mL of supernatant so that two Filter Units (and two Syringes) have to be used.
8.	Mix both filtrates and store deep-frozen.

7.3 Melatonin RIA

1.	Pipette 20 µL of Calibrator A into the tubes for the NSB and into the tubes for the unknowns .
2.	Pipette 20 µL of calibrators and controls into the respective tubes.
3.	Add 100 µL of Equalizing Reagent to tubes with NSB, calibrators and controls .
4.	Pipette 100 µL of unknowns into the respective tubes.
5.	Pipette 25 µL of Enzyme in all tubes (except totals) and vortex.
6.	Incubate for 1 hour at RT (20-25°C).
7.	Pipette 50 µL of Assay Buffer into all tubes (except totals) and mix shortly.
8.	Pipette 25 µL of Melatonin Antiserum into all tubes (except totals and NSB) ; mix thoroughly.
9.	Incubate for 1 hour at RT (20-25°C).
10.	Pipette 50 µL of the ¹²⁵ I Melatonin into all tubes and mix thoroughly.
11.	Cover tubes. Incubate for 20 - 24 hours at RT (20-25°C).
12.	Mix the chilled (2 - 8 °C) Precipitating Reagent thoroughly, pipette each 1 mL into all tubes (except totals) , and mix on a vortex.
13.	Incubate for 20 minutes at 2 - 8 °C .
14.	Centrifuge for 20 minutes at 3 000 x g , if possible in a refrigerated centrifuge.
15.	Decant or aspirate the supernatant carefully (except totals) . Blot the tubes dry and leave them upside for 2 minutes.
16.	Count all tubes for 1 minute in a gamma counter.

For volumes of unknown differing from 100 µL, the following modifications in reagent volumes are recommended:

Equalizing Reagent	Volume of unknown	Enzyme solution	Assay Buffer	Antiserum
100 µL	100 µL	25 µL	50 µL	25 µL
<i>25 µL</i>	<i>25 µL</i>	<i>25 µL</i>	<i>50 µL</i>	<i>25 µL</i>
<i>50 µL</i>	<i>50 µL</i>	<i>25 µL</i>	<i>50 µL</i>	<i>25 µL</i>
<i>200 µL</i>	<i>200 µL</i>	<i>50 µL</i>	<i>100 µL</i>	<i>50 µL</i>
<i>400 µL</i>	<i>400 µL</i>	<i>50 µL</i>	<i>100 µL</i>	<i>50 µL</i>

In principle the Melatonin Research RIA reagent kit will work for all kinds of biological fluids. However careful evaluation including recovery, linearity and sensitivity experiments is mandatory.

For the determination of Melatonin in rat unknowns follow the version with 50 µL volume of unknown.

8. Results

Subtract the mean cpm of the non-specific binding NSB from the mean cpm of calibrators, controls and unknowns.

The calibration curve from which the concentrations in the unknowns can be read off, is obtained by plotting the percentage of (B-NSB)/(B0-NSB) measured for the calibrators (linear, y-axis) against the corresponding calibrator concentrations (logarithmic, x-axis).

Use a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

The concentrations of the controls can be read directly from the respective calibration curve.

The concentrations of the unknowns are depending on the volume of the unknowns which is used for the assay and have to be **multiplied with a volume-factor**.

$$\text{Volume-factor} = \frac{20}{\text{volume which is used for the assay}}$$






Please use the following factors to correct for the volume and to determine the concentration of the calibrators [pg/mL]:

Volume of unknown	Equalizing Reagent	Volume Factor	Concentration of the calibrators(pg/ mL):						
			A	B	C	D	E	F	G
25 µL	25 µL	0.8	0	24	80	240	800	2 400	8 000
50 µL	50 µL	0.4	0	12	40	120	400	1 200	4 000
100 µL	100 µL	0.2	0	6	20	60	200	600	2 000
200 µL	200 µL	0.1	0	3	10	30	100	300	1 000
400 µL	400 µL	0.05	0	1.5	5	15	50	150	500

8.1 Quality control

It is recommended to use controls according to state and federal regulations. The kit, or other commercially available, controls should fall within established confidence limits. The confidence limits of the kit controls are printed on the QC-Report.

Symbols:

	Storage temperature				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!

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