novolytix

MELATONIN

RIA

For research use only.
Not for use in diagnostic procedures.

RK-MEL2-U 200 tests

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INTENDED USE

The Melatonin RadioImmunoAssay (RIA) is intended for the quantitative determination of melatonin (1-3) in serum, plasma, urine and other biological specimen **upon C18 solid phase extraction.**

PRINCIPLE OF THE ASSAY

Melatonin RIA kit measures melatonin by a double-antibody radioimmunoassay based on the Kennaway G280 anti-melatonin antibody (4)*. Reversed-phase column extracted samples and Controls and reconstituted Calibrators are incubated with the anti-melatonin antibody and ^{125}I-melatonin. ^{125}I-melatonin competes with melatonin present in samples, Calibrators and Controls. After 20 hours of incubation, solid-phase second antibody is added to the mixture in order to precipitate the antibody-bound fraction. After aspiration of the unbound fraction, the antibody bound fraction of ^{125}I-melatonin is counted.

* This publication uses a chloroform ($CHCl_3$) extraction of samples, prior to a ^{125}I-RIA and involves some different ancillary reagents besides the liquid phase second antibodies used in the report. Hence the performance specifications reported therein do not substitute those reported in this instructions.

REAGENTS SUPPLIED AND PREPARATION

Reagents	Quantity	Code	Reconstitution
Extraction Columns[1)] C18 reversed phase extraction columns 1 ml	20 pcs.	B-MEC	
Incubation Buffer	2 vials 100 ml	B-MEL-IB	Ready to use
Antiserum anti-melatonin antibody	2 vials 11 ml	B-MEL-AS	Ready to use
Tracer ^{125}I-melatonin	2 vials 11 ml	B-MEL-TR	Ready to use
Calibrator[2)] melatonin calibrators	1 set of 5 vials lyoph.	B-MEL-CASET	Reconstitute with 5 ml of Incubation Buffer
Controls Low / High[3)] melatonin in a protein buffer matrix	1 set of 2 vials 5.5 ml	B-MEL-CONSET	Ready for extraction
Second Antibody solid phase bound second antibody	2 vials 11 ml	B-AB2	Ready to use

Table 1

1) Each extraction column provided with this kit can be utilized up to five times if used according to the extraction procedures described in this instruction for use.
2) After reconstitution the Calibrators contain 0.5, 1.5, 5, 15 and 50 pg/ml of melatonin. **Reconstitute each calibrator with 5.0 ml of Incubation Buffer, vortex. Leave for at least 30 minutes at 2-8°C and vortex again.**
3) Lot specific amount of melatonin, see QC Data Sheet added to the kit. Extract the Controls according to the protocol as described on pp. 3ff.

STORAGE AND SHELF LIFE OF REAGENTS

Unopened reagents	
Store at 2-8°C. Do not use past kit expiration date. Columns should be stored at 18-28°C	
Opened / reconstituted reagents	
Extraction Columns	Used columns should be stored at 18-28°C and protected from dust and light.
Incubation Buffer	Stable at 2-8°C until expiration date printed on the label.
Antiserum	
Tracer	
Calibrators	Stable for at least 4 months at 2-8°C after reconstitution.
Controls	After extraction store Controls unreconstituted at -20°C
Second Antibody	Store refrigerated **(Do not freeze!)** Stable at 2-8°C until expiration date printed on the label.

Table 2

WARNINGS AND PRECAUTIONS

SAFETY PRECAUTIONS

- This kit contains an ionizing gamma-emitter with a half-life of 59.4 days (125-iodinated Melatonin; radionuclide is 125-Iodine, ^{125}I). The activity of the radioactive material in this kit does not exceed 74 kBq (2 µCi) of 125-Iodine.
- Receipt, acquisition, possession, use, and transfer are subject to the local regulations. Unused solutions and radioactive waste should be disposed of according to local State and Federal regulations.
- The Kit controls (B-MEL-CONSET) contain components of human origin. Although tested and found negative for HBV surface antigen, HCV and HIV1/2 antibodies, the reagents should be handled as if capable of transmitting infections and should be handled in accordance with good laboratory practices using appropriate precautions.

TECHNICAL PRECAUTIONS

- Read carefully the instructions prior to carrying out the test. Test performance will be adversely affected if reagents are incorrectly diluted, modified or stored under conditions other than those as detailed in this instruction for use.
- Incorrect results for standard curve, controls or samples may be obtained, if the 2nd antibody was not mixed sufficiently before pipetting. Do not freeze the 2nd antibody!
- Components must not be used after the expiry date printed on the labels.
- Do not mix different lots of reagents.
- Reconstitute the lyophilized reagents as indicated. Mix (vortex) well all reagents, particularly the Antiserum, and then let the reagents adjust to reach room temperature before use.
- Counting time should be selected in order to keep statistical counting error small: e.g., at 2000 cpm the counting error is at 5%; at 10000 cpm it is only 1%.
- If the initial concentration of an unknown sample reads above the highest calibrator, the sample should be further diluted with incubation buffer and tested again according to the assay procedure.

EQUIPMENT REQUIRED

- 100, 400, 1000 and 5000 µl precision pipettes (or preferably a 100–1000 µl adjustable multipipette) with disposable tip
- Extraction vacuum manifold for applying the extraction columns (optional)
- Vacuum concentrator or supply for particle free nitrogen
- Refrigerated centrifuge
- Vortex mixer
- Stir bar and magnetic stirrer
- Aspiration device
- Gamma-counter.

MATERIALS RECOMMENDED BUT NOT PROVIDED

- Disposable polystyrene tubes for the RIA (*e.g.* conical tubes from Sarstedt; no. 57.477)
- Disposable borosilicate glass tubes for the preparation of (serum) extracts (*e.g.* disposable CW glass test tubes from Baxter; no. 451296)
- Methanol *(HPLC grade)*
- Hexane *(p.a.)*
- Deionized double distilled water (ultrapure; not containing any organic residues).

SPECIMEN COLLECTION AND STORAGE

When drawing blood at night, a dim flash light or a yellow light (≤ 100 lux) should be used in order to avoid a possible light influence on the melatonin profile.

Serum: The procedure calls for about 2.5 ml of blood or for 1 ml of serum per extraction (if the sample is not diluted after extraction). Collect blood into plain tubes, avoid hemolysis, leave to clot for 45 min at room temperature (18-28°C) protected from light. Centrifuge at 1800 x g for 15 min at room temperature and collect the serum.
Lipemic, hemolytic and icteric samples should not be used in this assay. Lipemic samples can be avoided by asking patients to fast for at least 12 hours prior to drawing the.
Plasma: The procedure calls for about 2.5 ml of blood or for 1 ml of plasma per extraction (if the sample is not diluted after extraction). Collect blood into EDTA or Heparin tubes, centrifuge for 15 min at 2-8°C and 1800 x g, collect the plasma. Do not use grossly hemolysed samples.
Specimen Storage: If not extracted immediately, serum or plasma samples should be frozen and can be stored for at least 6 months at -20°C. Repeated freeze-thaw cycles should be avoided.

EXTRACTION OF SAMPLES AND CONTROLS

- Each extraction column provided with this kit can be used up to five times according to the extraction procedures described below. They should be stored at 18-28°C and protected from light and dust.
- Always use HPLC GRADE METHANOL AND HEXANE as well as DEIONIZED WATER OF ULTRAPURE QUALITY (no organic residues such as oils or detergents) for the extraction procedure.
- In order to avoid clogging of the columns, FILTER OR CENTRIFUGE SAMPLES CONTAINING PARTICLES such as fibrin clots prior to the extraction (*e.g.* heparin plasma that was frozen).
- The extraction method results in RECOVERIES of >90% with either ^{125}I-melatonin or ^{3}H-melatonin using human serum, plasma and urine samples.
- If samples have to be measured containing >50 pg/ml of melatonin, the sample volume may be reduced down to 0.125 ml without a notable change in extraction recoveries. This also indicates that SAMPLE VOLUMES AS SMALL AS 0.125 ML (≈ 0.3 ml of blood) may be used in this method (*cf.* page 4 for extractive dilution linearity).
- The extraction PROCEDURE WAS TESTED AND VALIDATED FOR HUMAN SERUM, PLASMA, SALIVA AND URINE samples. If you intend to measure any other specimen, it is recommended to validate the extraction recovery using ^{125}I-melatonin spiked specimens.

Extraction Procedure using Centrifugation

COLUMN PREPARATION & CONDITIONING

- Mark 1 extraction column for each sample to be extracted and place them into polypropylene or glass tubes.
- Add 1 ml of methanol to columns, centrifuge for 1 min at 200 x g.
  Repeat this step once.
- Add 1 ml of H_2O to columns, centrifuge for 1 min at 200 x g.
  Repeat this step once.
- Proceed with sample application without delay.

SAMPLE APPLICATION

- Add 1 ml of sample to the correspondingly marked column, centrifuge for 1 min at 200 x g.

WASHING

- Add 1 ml 10% methanol in H_2O (v/v) to the columns, centrifuge for 1 min at 500 x g.
  Repeat this step once.
- Add 1 ml of hexane to columns, centrifuge for 1 min at 500 x g.

ELUTION OF EXTRACT

- Place the columns into clean correspondingly marked borosilicate tubes.
- Add 1 ml of methanol to columns, centrifuge for 1 min at 200 x g.
- Use column for extracting the next sample (up to 5 times) or store column at 18-28°C
  Protect from light and dust.

EVAPORATION & RECONSTITUTION OF EXTRACT

- Evaporate the methanol to dryness using a vacuum concentrator with a cold trap. Alternatively, use a 37°C heating block or water bath and evaporate the methanol to dryness with a stream of particle free nitrogen.
- Reconstitute the samples with 1 ml of Incubation Buffer, vortex.
- Equilibrate the extracts for 30 min at 18-28°C, vortex. Store reconstituted extracts capped and frozen if not assayed immediately.

Extraction Procedure using Vacuum Manifold

Note: If not indicated otherwise, always pass the solvent through the column using vacuum and a flow rate of ≤ 5 ml/min.

COLUMN PREPARATION & CONDITIONING

- Mark 1 extraction column for each sample to be extracted and place them into polypropylene or glass tubes.
- Add 2 x 1 ml of methanol to columns, let the solvent pass through using vacuum.
- Add 2 x 1 ml of H_2O to columns, let the solvent pass through using vacuum.
- Proceed with sample application before the column gets dry.

SAMPLE APPLICATION

- Add 1 ml of sample to the correspondingly marked column, let the solvent pass through slowly (≤ 2 ml/min).
- Proceed with washing before the column gets dry.

WASHING

- Add 2 x 1 ml of 10% methanol in H_2O (v/v) to columns, let the solvent pass through using vacuum.
- Add 1 ml of hexane to the columns, let the solvent pass through using vacuum.
- Apply vacuum for 1 more min. in order to evaporate remaining hexane in the column.

ELUTION OF EXTRACT

- Place the columns into clean correspondingly marked borosilicate tubes.
- Add 1 ml of methanol to columns, let the solvent pass through slowly using vacuum and a flow rate of ≤ 2 ml/min.
- Use column for extracting the next sample (columns can be used up to 5 times) or store column at 18-28°C and protected from light and dust.

EVAPORATION & RECONSTITUTION OF THE EXTRACT

- Evaporate methanol to dryness using a vacuum concentrator with a cold trap. Alternatively, use a 37°C heating block or water bath and evaporate the methanol to dryness with a stream of particle free nitrogen.
- Reconstitute the samples with 1 ml of Incubation Buffer, vortex.
- Equilibrate the extracts for 30 min at 18-28°C, vortex. Store reconstituted extracts capped and frozen if not assayed immediately.

ASSAY PROCEDURE

1. Label 8 conical polystyrene tubes in duplicate: A to E for the Calibrator tubes, NSB (non-specific binding) for the blank tubes, MB for the maximum binding tubes and T for the total activity tubes. Label additional tubes in duplicate for patient samples and Controls.

2a. Pipet 500 µl of Incubation Buffer into the NSB tubes, and 400 µl of Incubation Buffer into the MB tubes.

2b. Pipet 400 µl of the Calibrators A to E into the corresponding tubes.

2c. Pipet 400 µl of the extracted patient samples and Controls into each of the correspondingly marked tubes.
3. Add 100 µl of the Antiserum to all tubes except the NSB and T tubes, vortex.
4. Add 100 µl of ^{125}I-melatonin tracer to all tubes. Vortex. Remove T tubes, they will need no further processing until counting at step 10.
5. Incubate all tubes for 20 (± 4) hours at 2-8°C.
6a. Invert the bottle containing the solid phase Second Antibody several times, add a stir bar and place the bottle on a magnetic stirrer.
6b. While stirring the Second Antibody suspension continuously, add 100 µl of the suspension to all assay tubes (except T tubes), vortex.
7. Incubate for 15 (± 2) min at 2-8°C.
8. Add 1 ml of cold, bidest. water to all assay tubes (except the T tubes).
9. Centrifuge for 2 min at 2000 x g and 2-8°C. Aspirate the supernatants (except T tubes) and retain the precipitates for counting.
10. Count the tubes for 2 min in a gamma-counter.

RESULTS & STANDARDIZATION

- Record the cpm for all tubes (T, NSB, MB, A, B, C, D, E-Calibrators 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 = $cpm_{Average}$ - $cpm_{Average\ NSB}$

1. 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%.

$$\text{percent bound} = \frac{\text{net cpm}}{\text{net MB cpm}} \times 100$$

2. Prepare a lin/log graph paper and plot the percent bound on the vertical axis against the melatonin concentration (pg/ml) on the horizontal axis for each of the Calibrators. Draw the best fitting curve or calculate the standard curve using a four-parameter logistic (4-PL), a spline smoothed or an equivalent algorithm.
3. Determine melatonin concentrations for the patient samples and Controls from this standard curve. Alternative data reduction methods are equally acceptable.

See Table 3 and Figure 1 for examples of a standard curve. This standard curve is for demonstration purposes only. A standard curve must be generated for each set of samples to be assayed.

Standardization: MELATONIN RIA is calibrated with UV/VIS: ε_{278} = 6300 $M^{-1}cm^{-1}$ in ethanol.

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 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.

LIMTATIONS

Melatonin results should be interpreted in conjunction with information available from clinical assessment of the patient and other diagnostic procedures.

PERFORMANCE CHARACTERISTICS

The assay performance characteristics have been validated in duplicates.

Intra-Assay Precision (Run-to-Run) of RIA: 7.9%. The intra-assay precision was calculated from results of 20 pairs of values from each sample in a single run. The results are presented in Table 4.

Inter-Assay Precision (Run-to-Run) of RIA: 11.7%. The inter-assay precision was calculated from results of 20 pairs of values in 20 different runs. The results are presented in Table 5.

Intra-Assay Precision (Run-to-Run) of Column Extraction and RIA combined: 8.2%. A daytime human serum was extracted 12 times in parallel using 12 separate extraction columns. Subsequently, all extracts were analyzed in a single run according to the assay procedure. The results are presented in Table 6.

Detection Limit (LoB): 0.3 pg/ml. Twenty Zero Calibrator (MB) replicates were assayed in a single run. The minimum detectable concentration of melatonin in 400 ml of unextracted Incubation Buffer was calculated to be 0.3 pg/ml (1.3 pmol/l) by subtracting 2 standard deviations of averaged Zero Calibrator duplicates from the counts at maximum binding and intersecting this value with the standard curve obtained in the same run.

Detection Limit (LoQ): 0.9 pg/ml. The functional least detectable dose (Limit of Quantitation) was calculated to be 0.9 pg/ml (cut-off of intra-assay CV = 10%).

Dilution/Linearity-Parallelism: 103.7%. Two human serum samples containing an elevated concentration of elevated concentration of melatonin was extracted both, diluted and undiluted with Incubation Buffer and subsequently assayed according to the assay procedure. The results are presented in Table 7.

Extractive Dilution Linearity: 108.8 %. Varying amounts of a human serum sample containing an elevated concentration of melatonin were applied onto extraction columns, extracted according to the protocol and subsequently assayed according to the assay procedure. The results are presented in Table 8.

Extraction Recovery: 99.9 %. Two serum samples were spiked with increasing amounts of melatonin, extracted and analyzed according to the assay procedure. The results are presented in Table 9.

Specificity: In Table 10 the crossreactivities of the melatonin antiserum were found at 50 % binding.

Table 3 **Expamples of results**

	cpm	B/T [%]	B/B_0 [%]	Conc. [pg/ml]	cpm_{CV} [%]
Total	17145.9	100.0			
Total	16942.3	100.0			
Total Avg	**17044.1**	**100.0**			**0.8**
NSB	479.1	2.81			
NSB	434.6	2.55			
NSB Avg	**456.9**	**2.68**			**6.9**
MB	5501.7	32.28	100.0		
MB	5478.6	32.14	100.0		
MB Avg	**5490.2**	**32.21**	**100.0**		**0.8**
Cal A	5029.2	29.51	91.0		
Cal A	5020.7	29.46	90.9		
Cal A Avg	**5025.0**	**29.48**	**90.9**	**0.5**	**0.1**
Cal B	4218.6	24.75	74.9		
Cal B	4285.2	25.14	76.2		
Cal B Avg	**4251.9**	**24.95**	**75.5**	**1.5**	**1.1**
Cal C	3020.9	17.72	51.0		
Cal C	2949.6	17.31	49.6		
Cal C Avg	**2985.3**	**17.51**	**50.3**	**5.0**	**1.7**
Cal D	1921.1	11.27	29.1		
Cal D	1866.2	10.95	28.1		
Cal D Avg	**1893.6**	**11.11**	**28.6**	**15.0**	**2.1**
Cal E	950.6	5.58	9.8		
Cal E	1006.9	5.91	10.9.		
Cal E Avg	**978.8**	**5.74**	**10.4**	**50.0**	**4.1**
Con L	3666.3		63.8	2.69	
Con L	3669.6		63.8	2.68	
Con L Avg	**3668.0**		**63.8**	**2.69**	**0.2**
Con H	1607.7		22.9	21.20	
Con H	1580.4		22.3	21.96	
Con H Avg	**1594.1**		**22.6**	**21.58**	**2.5**

ED_{20} = 25.55 pg/ml ED_{50} = 5.04 pg/ml ED_{80} = 1.14 pg/ml

Figure 1 **Example of Standard Curve**

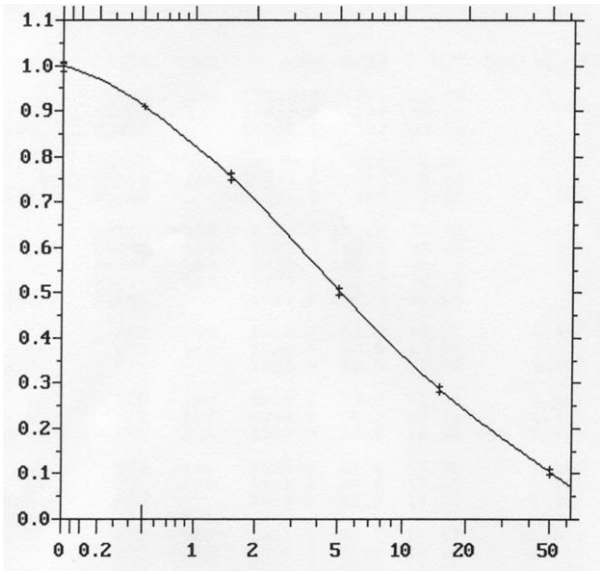

Table 4 **Intra Assay Precision**

Sample	Mean [pg/ml]	SD [pg/ml]	CV [%]
Low Control	4.6	0.31	6.7
High Control	14.3	1.08	7.5
Low Serum	1.9	0.19	10.0
High Serum	21.9	1.58	7.2
Mean			7.9

Table 5 **Inter-Assay Precision**

Sample	Mean [pg/ml]	SD [pg/ml]	CV [%]
Low Control	2.3	0.38	16.2
High Control	18.7	1.35	7.2
Mean			11.7

Table 6 **Intra-Assay Precision Column Extraction and RIA**

Sample	Mean [pg/ml]	SD [pg/ml]	CV [%]
Low Serum1	1.6	0.22	14.0
Low Serum2	2.8	0.17	6.1
High Serum	19.5	0.88	4.5
Mean			8.2

Table 7 **Dilution/Liniearity-Parallelism**

Sample	Dilution	Obs. (O) [pg/ml]	Exp. (E) [pg/ml]	% O/E
High Control	1:1	18.9	-	-
	1:2	9.6	9.5	101.4
	1:4	5.2	4.7	109.0
	1:8	2.7	2.4	114.3
	1:16	1.4	1.2	119.7
High Serum	1:1	36.3	-	-
	1:2	16.8	18.2	92.7
	1:4	8.3	9.1	91.6
	1:8	4.3	4.5	95.4
	1:16	2.3	2.3	100.7
	1:32	1.2	1.1	108.3
Mean				103.7

Table 8 **Extractive Dilution-Linearity**

Sample	Volume applied [ml]	Obs. (O) [pg/ml]	Exp. (E) [pg/ml]	% O/E
High Serum	1	29.1	-	-
	0.5	15.1	14.6	103.8
	0.25	7.8	7.28	107.2
	0.125	4.2	3.64	115.4
Mean				108.8

Table 9 **Extraction Recovery**

Sample	Basic Value	Added [pg/ml]	Observed [pg/ml]	Expected [pg/ml]	Recovery [%]
A	0.46	1	1.2	1.5	83
		2	2.2	2.5	88
		5	5.2	5.5	95
		10	10.5	10.5	100
		20	21.7	20.5	106
		40	41.0	40.5	101
B	0.61	1	1.9	1.6	120
		2	2.3	2.6	87
		5	5.5	5.6	98
		10	11.8	10.6	111
		20	22.1	20.6	107
		40	41.3	40.6	102
Mean					99.9

Table 10 **Specificity**

Compound	Crossreactivity [%]
melatonin	100
6-sulfatoxymelatonin	0.002
serotonin	< 0.001
5-hydroxy-indoleacetic acid	< 0.001
N-acetylserotonin	0.027
5-methoxytryptamine	0.003
5-methoxytryptophan	0.001
5-methoxytryptophol	0.001

Table description: cf. “Results” and “Performance Characteristics” (page 4).

APPENDIX II
REFERENCES

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2. Luboshitzky R. et al. *Daily and seasonal variations in the concentration of melatonin in the human pineal gland*. Brain Res Bull 47, 271-6 (1998)
3. Danilenko K. et al. *Phase advance after one or three simulated dawns in humans*. Chronobiol Int 17, 659-68 (2000)
4. Vaughan G M: *New sensitive serum melatonin radioimmunoassay employing the Kennaway G280 antibody: Syrian hamster morning adrenergic response*. J Pineal Res 15, 88-103 (1993).

APPENDIX III
SHORT PROTOCOL

EXTRACTION PROCEDURE

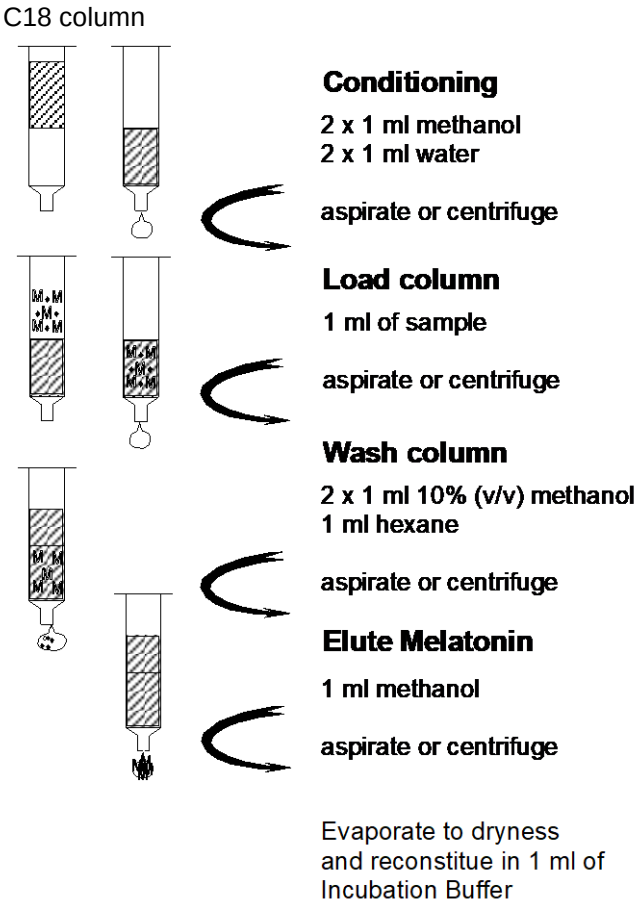

Figure 2

RADIOIMMUNOASSAY PROCEDURE

Polystyrene tubes in duplicate	Incubation Buffer (µl)	Calibrator, Control, Sample (µl)	Antiserum (µl)	Tracer (µl)		Second Antibody (µl)	
Total	--	--	--	100		--	**Vortex and incubate for 15 minutes(± 1 min) at 2-8°C**
NSB	500	--	--	100		100	
MB	400	--	100	100		100	**add 1 ml of deionized water (except T tubes) and centrifuge for 2 minutes at 2-8°C and 2000 x g**
Std A 0.5 pg/ml	--	400	100	100		100	
Std B 1.5 pg/ml	--	400	100	100		100	
Std C 5.0 pg/ml	--	400	100	100	**Vortex and incubate at 2-8°C for 20 hours (± 4 hours)**	100	
Std D 15.0 pg/ml	--	400	100	100		100	
Std E 50.0 pg/ml	--	400	100	100		100	**aspirate supernatant (except T tubes) and count for 2 minutes in a Gamma counter**
Sample	--	400	100	100		100	

Table 11

Symbol	Explanation
	Use by Verwendbar bis Utiliser jusqu'au Utilizzare entro Fecha de caducidad
REF	Catalogue number Bestellnummer Référence du catalogue Numero di catalogo Número de catálogo
LOT	Batch code Chargenbezeichnung Code du lot Codice del lotto Codigo de lote
IVD	*In Vitro* Diagnostic Medical Device *In Vitro* Diagnostikum Dispositif médical de diagnostic *in vitro* Dispositivo medico-diagnostico *in vitro* Producto sanitario para diagnóstico *in vitro*
	Temperature limitation Zulässiger Temperaturbereich Limites de température Limiti di temperatura Limite de temperatura
	Consult Instructions for Use- Gebrauchsanweisung beachten Consulter le mode d'emploi Consultare le istruzioni per l'uso Consulte las instrucciones de uso
	Contains sufficient for <n> tests Ausreichend für „n" Ansätze Contenu suffisant pour „n" tests Contenuto sufficiente per „n" saggi Contenido suficiente para <n> ensayos
	Radioactive Material Radioaktives Material Matériel radioactif Materiale radioattivo Material radiactivo

Symbol	Explanation
MEC	Extraction Columns Extraktionssäulen Colonne d'extraction Colonne d' estrazione Columnas de extracción
BUF INC	Incubation Buffer Inkubations-Puffer Tampon d'incubation Tampone d'incubazione Tampón de incubación
Ab	Antiserum Antiserum Antisérum Antisiero Antisuero
TR	Tracer Tracer Traceur Tracciante Trazador
CAL A - CAL E	Calibrator A - E Kalibrator A - E Calibrateur A - E Calibratore A - E Calibrador A - E
CONTROL L	Control Low Kontrolle tief Contrôle bas Controllo basso Control bajo
CONTROL H	Control High Kontrolle hoch Contrôle élevé Controllo alto Control alto
Ab2	2nd Antibody 2. Antikörper 2ème Anticorps Secondo anticorpo Segundo anticuerpo

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