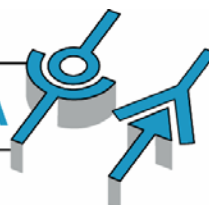


**Instructions for use**  
**EURIA-MOTILIN**

**EURO-DIAGNOSTICA**



# **EURIA-MOTILIN**

Motilin radioimmunoassay  
(Cat. No. RB 308)  
100 tubes  
For research use only

Doc. no. E-23-0031-02  
January, 2006

## **INTRODUCTION**

Motilin is a straight chain peptide containing 22 amino acids and has a molecular weight of approximately 2700. Motilin was originally isolated from the small intestine.

Motilin has been shown to be contained in cytoplasmic secretory granules in endocrine cells located in the epithelium of the duodenum and jejunum.

Motilin induces contraction of intestinal smooth muscle. Motilin stimulates gastric pepsin secretion, lower esophageal sphincter pressure, and contraction of the gallbladder.

Fluctuations in circulating plasma motilin levels have been shown to correlate with intestinal motility events.

## **PRINCIPLE OF THE METHOD**

The intended use of these reagents is for the assay of motilin in human serum or tissue extracts. Motilin in the samples is assayed by the competitive radioimmunoassay using rabbit antibodies to synthetic, porcine motilin. Motilin in standards and samples compete with <sup>125</sup>I-labelled motilin in binding to the antibodies. <sup>125</sup>I-motilin binds in a reverse proportion to the concentration of motilin in standards and samples. In order to increase the sensitivity of the assay a sequential incubation with delayed addition of <sup>125</sup>I-motilin is performed. Antibody bound <sup>125</sup>I-motilin is separated from the unbound fraction by using the double antibody polyethylene glycol precipitation technique. The radioactivity of the precipitates is measured.

## PRECAUTIONS

For in vitro use only.

As the regulations may vary from one country to another, it is essential that the person responsible for the laboratory is familiar with current local regulations, concerning all aspects of radioactive materials of the type and quantity used in this test.

This kit contains components of human origin. They have been tested by immunoassay for hepatitis B surface antigen, antibodies to HCV and for antibodies to HIV-1 and HIV-2 and found to be negative. Nevertheless, all recommended precautions for the handling of blood derivatives should be observed.

Steps should be taken to ensure the proper handling of the radioactive material, according to local and/or national regulations. Only authorized personnel should have access to the reagents.

The following precautions should be observed when handling radioactive materials:

- Radioactive material should be stored in specially designated areas, not normally accessible to unauthorized personnel.
- Handling of radioactive material should be conducted in authorized areas only.
- Care should be exercised to prevent ingestion and contact with the skin and clothing. Do not pipette radioactive solutions by mouth.
- Drinking, eating or smoking should be prohibited where radioactive material is being used.
- Hands should be protected by gloves and washed after using radioactive materials.
- Work should be carried out on a surface covered by disposable absorbing material.
- Spills of radioactive material should be removed immediately, and all contaminated materials disposed as radioactive waste. Contaminated surfaces should be cleaned with a detergent.

The reagents in this kit contain sodium azide. Contact with copper or lead drainpipes may result in the cumulative formation of highly explosive azide deposits. On disposal of the reagents in the sewerage, always flush with copious amounts of water, which prevents metallic azide formation. Plumbing suspected of being contaminated with these explosive deposits should be rinsed thoroughly with 10% sodium hydroxide solution.

## COMPOSITION OF THE REAGENT KIT

The reagents provided in each kit are sufficient for 100 tubes.

### 1. Anti-motilin (Reagent A)

Rabbit antiserum raised against synthetic porcine motilin. Lyophilized in 2.0 mL 0.5 M phosphate buffer, pH 7.4, containing 2.5% human serum albumin, 2.5% EDTA, disodium salt, 0.5% sodium azide and 5000 KIU Trasylol<sup>®</sup>/mL. For 100 tubes. Reconstitution in 22 mL distilled water.

### 2. <sup>125</sup>I-Motilin (Reagent B)

Contains 1.5 µCi or 56 Kbcq at reference date. Synthetic motilin is labelled with <sup>125</sup>I by the chloramine-T method and HPLC-purified, monoiodinated.

Specific activity: 1700-2100 µCi/nmol (62-77 MBq/nmol). Lyophilized in 2.5 mL 0.5M phosphate buffer, pH 7.4, with 2.5% human serum albumin, 2.5% EDTA disodium salt, 0.5% sodium azide and 5000 KIU Trasylol<sup>®</sup>/mL. Contains 0.12 mL normal rabbit serum. Reconstitution in 25 mL distilled water.

### 3. Double antibody-PEG (Reagent C)

50 mL diluted goat anti-rabbit Ig antiserum in 0.05 M phosphate buffer, pH 7.4, 0.25% human serum albumin, 0.25% EDTA disodium salt and 0.05% sodium azide.

Contains 5.0% (w/v) polyethylene glycol 6000.

### 4. Diluent (Reagent D)

25 mL 0.05 M phosphate buffer, pH 7.4, with 0.25% human serum albumin, 0.25% EDTA disodium salt, 0.05% sodium azide and 500 KIU Trasylol<sup>®</sup>/mL. Inteded for dilution of the motilin standard and replacement of the antiserum in non-specific binding controls.

### 5. Motilin standard 1000 pmol/L (Reagent E)

5.00 mL, 1000 pmol/L, lyophilized. Produced from synthetic, porcine motilin. The peptide was dissolved in 0.05 M phosphate buffer, pH 7.4, with 0.25% human serum albumin, 0.25% EDTA disodium salt, 0.05% sodium azide and 500 KIU Trasylol<sup>®</sup>/mL and lyophilized. Reconstitution with 5.00 mL distilled water.

### 6. Controls (Reagent F-G)

Lyophilized controls with two different levels of motilin. 1.00 mL of each control after reconstitution. The motilin concentrations are given on the labels of the vials.

Contains 0.05% sodium azide.

## **EQUIPMENT AND REAGENTS REQUIRED BUT NOT PROVIDED**

Distilled water.

11-13 x 55 mm disposable tubes, polystyrene.

Pipettes glass: 1.00, 2.00, 5.00 mL.

Pipettes with disposable tips, 100, 200 and 500  $\mu$ l.

Measuring cylinder: 25 mL.

Vortex mixer.

Refrigerator.

Centrifuge, refrigerated, giving minimum 1700 x g.

Gamma counter.

## REAGENT PREPARATION AND STORAGE

Store all reagents at 2-8° C before reconstitution and use. The water used for reconstitution of lyophilized reagents should be distilled in an all-glass apparatus or be of corresponding purity. Dissolve the content of a vial by gentle inversion and avoid foaming. The stability of the reagents is indicated on the labels of the vials. For lyophilized reagents the expiry date is valid for the unreconstituted reagents. The reconstituted reagents are stable for 10 weeks if stored properly.

### **Reagent A: Anti-motilin**

Reconstitute with 22 mL distilled water.

Store at 2-8° C.

### **Reagent B: <sup>125</sup>I-motilin**

Reconstitute with 25 mL distilled water.

Store at -18° C or lower if reused.

### **Reagent C: Double antibody-PEG**

Ready for use. Mix thoroughly before use.

Store at 2-8° C.

### **Reagent D: Diluent**

Ready for use.

Store at 2-8° C.

### **Reagent E: Motilin standard, 1000 pmol/L**

Reconstitute with 5.00 mL distilled water. For preparation of working standards see assay procedure.

Store at -18° C or lower if reused.

### **Reagent F-G: Controls**

Reconstitute each vial with 1.00 mL distilled water. Store at -18° C or lower if reused.

## SPECIMEN COLLECTION

Vein blood is collected without additives. The sample is cooled in an ice-bath immediately. Serum is separated by centrifugation at +4° C. The serum should be frozen within 2 hours and stored at -18° C or lower until assayed. Repeated freezing and thawing should be avoided.

## ASSAY PROCEDURE

For an overview see page 12.

Accuracy in all pipetting steps is essential. All tests (standards, controls and samples) should be performed in duplicate.

A complete assay includes:

**Standards (St-tubes):** 7 different concentrations, 0, 15.6, 31.3, 62.5, 125, 250 and 500 pmol/L.

**Samples (S-tubes):** Unknown samples.

**Controls (C-tubes):** Low and high control

Tubes for determination of the *non-specific binding (NSB-tubes)*.

Tubes for determination of the *total radioactivity (TOT-tubes)*.

## PERFORMANCE

1. Reconstitute the reagents according to the instructions.
2. Prepare the motilin working standards by dilution of the 1000 pmol/L standard (Reagent E) with the diluent (Reagent D) according to the following:
  - a/ 1.00 mL standard 1000 pmol/L + 1.00 mL diluent = 500 pmol/L.
  - b/ 1.00 mL standard 500 pmol/L + 1.00 mL diluent = 250 pmol/L.
  - c/ 1.00 mL standard 250 pmol/L + 1.00 mL diluent = 125 pmol/L.
  - d/ 1.00 mL standard 125 pmol/L + 1.00 mL diluent = 62.5 pmol/L.
  - e/ 1.00 mL standard 62.5 pmol/L + 1.00 mL diluent = 31.3 pmol/L
  - f/ 1.00 mL standard 31.3 pmol/L + 1.00 mL diluent = 15.6 pmol/L
  - g/ Diluent = 0 nmol/L.

Store at -18° C.
3. Pipette 100 µL of standards a-g (0-500 pmol/L), samples and controls in their respective tubes. Pipette 100 µL of the zero-standard in the NSB-tubes.
4. Add 200 µL anti-Motilin (Reagent A) to all tubes except the NSB- and TOT-tubes.
5. Add 200 µL assay diluent (Reagent D) to the NSB-tubes.
6. Vortex-mix and incubate for 18-24 hours at 2-8° C.
7. Pipette 200 µL <sup>125</sup>I-motilin (Reagent B) into all tubes. The TOT-tubes are sealed and kept aside.
8. Vortex-mix and incubate for 18-24 hours at 2-8° C.
9. Add 500 µL well mixed double antibody-PEG (Reagent C) to all tubes except the TOT-tubes.
10. Vortex-mix and incubate for 30-60 minutes at 2-8° C.
11. Centrifuge the tubes for 15 minutes at +4° C (1700 x g).
12. Decant the supernatant immediately after centrifugation.
13. Count the radioactivity of the precipitates and the TOT-tubes in a gamma counter (counting time 1-2 minutes).

## CALCULATION OF RESULTS

1. Subtract the average count rate (CPM) of the non-specific binding from the count rate (CPM) of the replicates of the standards, controls and samples.
2. A standard curve is generated by plotting the precipitated CPM bound fraction (in cpm or % B/TOT) against the concentrations of the motilin standards. An example of a standard curve is given on page 13.
3. Interpolate the motilin concentrations in the samples and controls from the generated standard curve.
4. The standard curve and the calculation of the concentrations in the samples can also be done by a computer method. A spline method may be used.

## ASSAY CHARACTERISTICS

### Sensitivity

The sensitivity calculated from a decrease in binding of 2 SD in the zero standard is 10 pmol/L.

### Precision

*Intra assay variation*

<u>Level</u>	<u>Coefficient of variation (%CV)</u>
100 pmol/L	4.0%
250 pmol/L	4.3%

*Between assay variation (total variation)*

<u>Level</u>	<u>Coefficient of variation (%CV)</u>
100 pmol/L	5.6%
250 pmol/L	6.1%

### Specificity

The following results were obtained in cross reactivity studies with other polypeptides:

<u>Polypeptide</u>	<u>Cross reaction</u>
Motilin, porcine	100.0%
Gastrin-17, human	<0.01%
Secretin, porcine	0.07%
Cholecystokinin 1-39, porcine	<0.01%
Vasoactive intestinal peptide, human	<0.01%
Gastric inhibitory peptide, porcine	<0.01%
$\beta$ -endorphin, human	<0.01%
Neuropeptide Y, human	<0.01%
Neuropeptide YY, human	<0.01%

## MOTILIN IN HUMAN SERUM

The motilin concentration was measured in serum from overnight fasting individuals.  
Found: <110 pmol/L.

## QUALITY CONTROL

In order to enable the laboratory to completely monitor the consistent performance of the assay, the following important factors should be checked.

**1. Controls**

The found concentrations of the controls (reagent F-G) should be within the limits given on the labels of the vials.

**2. Total counts**

Counts obtained should approximate the expected CPM when adjusted for counter efficiency and radioactive decay. The content of <sup>125</sup>I-Motilin in this kit will give a total counts in the assay (TOT) of 21000 CPM (+20%, -5%) at the activity reference date (counting efficiency = 80%).

**3. Maximum binding (Bo/TOT)**

Calculate for each assay the % bound radioactivity in the zero-standard:

$$\left(\frac{\text{Bo}}{\text{TOT}} \times 100\right)$$

$\frac{\text{Bo}}{\text{TOT}} \times 100$  is generally 40-60% at the activity reference date

**4. Non-specific binding (NSB/TOT).**

Calculate for each assay the non-specific binding:  $\left(\frac{\text{NSB}}{\text{TOT}} \times 100\right)$

$\frac{\text{NSB}}{\text{TOT}} \times 100$  is less than 6% if decanting is made properly.

**5. Shape of standard curve.**

For example, monitor the 80, 50 and 20% points of the standard curve for run to run reproducibility.

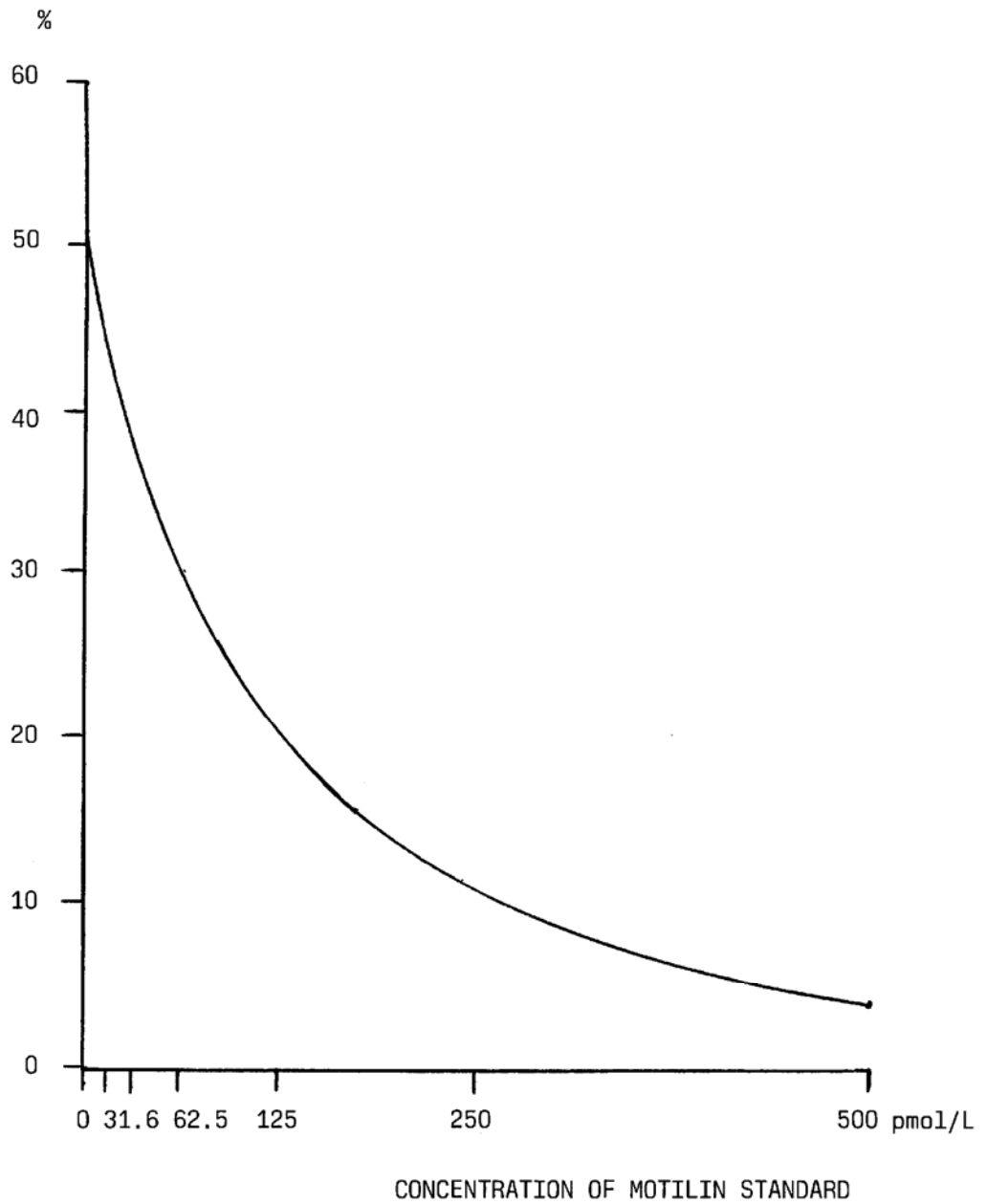
## OUTLINE OF THE RIA PROCEDURE

Type of tubes	Tube no	Standard sample or control	Anti-motilin (A)	Diluent (D)		<sup>125</sup> I-motilin (B)		Double antibody PEG (C)	
TOT	1-2	-	-	-	Vortex-mix and incubate for 18-24 hours at 2-8° C.	200 µL	Vortex-mix and incubate for 18-24 hours at 2-8° C.	-	Vortex-mix and incubate for 30-60 min. at 2-8° C. Centrifuge 15 min. at 1700 x g at +4° C. Decant and count the radioactivity of the precipitates.
NSB, stand 0	3-4	100 µL	-	200		200 µL		500 µL	
Stand 0	5-6	100 µL	200 µL	-		200 µL		500 µL	
Stand 15.6	7-8	100 µL	200 µL	-		200 µL		500 µL	
Stand 31.3	9-10	100 µL	200 µL	-		200 µL		500 µL	
Stand 62.5	11-12	100 µL	200 µL	-		200 µL		500 µL	
Stand 125	13-14	100 µL	200 µL	-		200 µL		500 µL	
Stand 250	15-16	100 µL	200 µL	-		200 µL		500 µL	
Stand 500	17-18	100 µL	200 µL	-		200 µL		500 µL	
Control F	19-20	100 µL	200 µL	-		200 µL		500 µL	
Control G	21-22	100 µL	200 µL	-		200 µL		500 µL	
Sample 1	23-24	100 µL	200 µL	-		200 µL		500 µL	
Sample 2	25-26	100 µL	200 µL	-	200 µL	500 µL			

EXAMPLE OF MOTILIN STANDARD CURVE

$$\frac{B}{TOT} \times 100$$

(corrected for NSB)



## REFERENCES

1. Sjölund, K., Ekman, R., Akre, F. and Lindner, P.  
Motilin in chronic idiopathic constipation.  
Scand J Gastroenterol 21:914-918 (1986).
2. Hansson, M., Almer, L.D., Ekman, R., Janzon, L. and Trelle, E.  
Motilin response to a glucose load aberrant in smokers.  
Scand J Gastroenterol 22:809-812 (1987).
3. Lothe, L., Ivarsson, S-A., Ekman, R. and Lindberg, T.  
Motilin and infantile colic, a prospective study.  
Acta Paed Scand 79:410-416 (1990).
4. Brown, J.C., Mutt, V. and Bryburgh, J.R.  
The further purification of motilin, a gastric motor activity stimulating polypeptide from the mucosa of the small intestine of hogs.  
Can J Physiol Pharmacol 49:399 (1971).
5. Brown, J.C., Cook, M.A. and Dryburgh, J.R.  
Motilin, a gastric motor activity-stimulating polypeptide: Final purification, aminoacid composition, and C-terminal residues.  
Gastroenterology 62:401 (1972).
6. Itoh, Z., Honda, R., Hiwatashi, K. et al.  
Motilin-induced mechanical activity in the canine alimentary tract.  
Scand J Gastroenterol 11: (suppl 39) 110 (1976).
7. Wingate, D.L., Ruppin, H., Green, W.E.R. et al.  
Motilin-induced electrical activity in the canine gastrointestinal tract.  
Scand J Gastroenterol 11: (Suppl 39) 111 (1976)
8. Poitras, P., Steinbach, J.H., Vandeventer, G. et al.  
Motilin independent ectopic fronts of the interdigestive myoelectric complex in dogs.  
Am J Physiol 239:G 215 (1980).
9. Sarna, S., Chey, W.Y., Condon, R.E., et al.  
The cause and effect relationship between motilin and migrating myoelectric complex.  
Am J Physiol 245:G 277 (1983).
10. Poitras, P.  
Motilin is a digestive hormone in the dog.  
Gastroenterology 87:909 (1984).
11. Hall, K.E., Greenberger, G.R., El-Sharkaway, T.Y. and Diamant, N.E.  
Relationship between porcine motilin-induced migrating motor complex-like activity, vagal integrity, and endogenous motilin release in dogs.  
Gastroenterology 87:76 (1984).

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