**Revised 17 June 2010 rm (Vers. 2.0)** **For Veterinary Use Only**

***Please use only the valid version of the package insert provided with the kit.***

FOR RESEARCH USE ONLY

## Intended use

Enzyme Linked Immunosorbent assay for the IN VITRO determination of prolactin in rat serum and plasma (rPRL).

## Principle of the method

The ELISA technique uses antibodies with high affinity and specificity for two different epitopes on rat prolactin. A first mouse anti-rPRL monoclonal antibody bound to a polystyrene well will capture the rPRL of the sample in the presence ofa second Horseradish peroxidase conjugated mouse anti-rPRL monoclonal antibody.
Following the incubation and the one step formation of the solid phase – rPRL -conjugated monoclonal antibody sandwich, the well is washed to remove excess of unbound conjugated antibody. Then the chromogen/substrate is added, which turns from pink to blue proportionally to rat prolactin (rPRL) concentration in the sample. Addition of the stop solution turns the color to yellow. The intensity of the yellow color is measured using a spectrophotometer with a 450 nm filter. Sample concentrations are read from a calibration curve and the results are expressed in ng/ml.

## Warnings and precautions

For **in vitro use only.**
It must be handled by specialized staff.
Good laboratory and safety practices are advisable.
***Warning*** : this kits contains TMB substrate(3,3',5,5' tetramethylbenzidine) which has shown a possible mutagenic effect in experimental animals (mice).
Avoid contact with skin and eyes. May give irritation of eyes, skin and gastrointestinal.
***Warning*** : Animal origin materials are used in this kit, these are provided with sanitary certificate. However, no known test can guarantee that such material does not contain any infectious agents. These products must be considered as potentially infectious and handled with care.

## Reagents, preparation and storage

All reagents are ready for use, except the washing solution, calibrators and controls (1-2).
Stored at 2-8°C, the material can be used up to the expiration date printed on each label.
The diluted washing solution can be stored at 2-8°C or 18-25°C.

Before use, reconstitute the content of the calibrator (CAL) with 3 ml of diluent buffer (BUF) and the content of controls (1-2) with 0.5 ml of diluent buffer (BUF). Mix gently to avoid foaming. Wait at least 15 minutes after solubilization before dispensing.
If not used immediately after reconstitution, store aliquots at –20°C for up to 4 weeks.
After use, close all reagents vials and bottles and replace these at 2-8°C or –20°C. Store the unused strips/wells with the dessicant sachet in the provided minigrip bag at 2-8°C. Do not forget to reseal the bag.

**Preparation of the calibrators 0-7**

| | Volume of calibrator (µl) | Volume of buffer solution (BUF) | Conc. ng/ml |
|---|---|---|---|
| CAL 7 | CAL (see 4.3) | 3000 µl | 180.00 |
| CAL 6 | 500 µl of CAL 7 | 500 µl | 90.00 |
| CAL 5 | 200 µl of CAL 7 | 600 µl | 45.00 |
| CAL 4 | 100 µl of CAL 7 | 900 µl | 18.00 |
| CAL 3 | 50 µl of CAL 7 | 950 µl | 9.00 |
| CAL 2 | 25 µl of CAL 7 | 975 µl | 4.50 |
| CAL 1 | 25 µl of CAL 7 | 1975 µl | 2.25 |
| CAL 0 | - | 2000 µl | 0.00 |

**Example of dilution scheme.**
**The exact concentrations of rat prolactin calibrator (CAL) is printed on the vial label.**

4.1. **SORB MAb PRL**
96 breakable wells polystyrene microplate coated with mouse anti - rat prolactin monoclonal antibody. Systematically allow the microwells/microplate to reach room temperature before opening the bag. Single use strips/wells.

4.2. **CONJ ENZ**
2 bottles (12 ml/bottle, yellow) Horseradish peroxydase (HRP) conjugated mouse anti – rat prolactin monoclonal antibody diluted in buffer containing a yellow dye.

4.3. **CAL**
1 vial rat prolactin lyophilized in buffer. The calibrator is calibrated against internal reference preparation of rat prolactin. The concentration expressed in ng/ml of the calibrator is printed on the vial label.

4.4. **CONTROL 1-2**
2 vials vial rat prolactin lyophilized in buffer. The controls have to be assayed with the samples and the results compared with those printed on the vials

4.5. **BUF**
2 bottles (10 ml/bottle) of diluent buffer containing preservative (Proclin 0.05%).

4.6. **SUBS TMB**
2 bottles (12 ml/bottle) TMB substrate (tetramethyl benzidine) in buffer containing a pink dye.
**Light sensitive, protect from light.**

4.7. **SOLN STOP**
1 bottle (10 ml) $H_2SO_4$ 2M.

4.8. **BUF WASH 20x**
1 bottle (60 ml) concentrated buffer solution containing preservative (Proclin 0.05%). Poor the solution in 1140 ml of distilled water and homogenize.

**Material required but not provided**

- Test tubes for the dilutions and a tube holder
- Vortex mixer
- Manual or automated precision micropipettes with single use tips for dispensing samples or reagents without cross-contamination.
- Multichannel micropipette or repeating dispenser (Eppendorf type)
- Vacuum pump connected through a trap for aspiration
- 96-well microplate reader with a 450 nm filter
- Semi-logarithmic paper (or software package)
- Microplate washer (facultative).

**Methodology**

***Collection and handling of serum or plasma samples***

The blood sample may be collected either into a dry tube or one containing an anticoagulant. If heparin is used, only the minimum required to avoid clotting should be added.

The serum or plasma when separated from the red blood cells, may be assayed immediately, within 24 hours if stored at 2-8°C, or after periods up to several months if stored at -20°C.

Repeating freezing and thawing must be avoided.

***Assay Procedure***

**Do not mix reagents of different lots**
**Bring the different components of the kit to room temperature prior to use. Perform the assay in duplicates. Calibrators, controls and samples must be assayed at the same time. Follow strictly the different steps of the procedure and use interchangeable tips**

Select the number of coated wells for rat prolactin assays. Replace unrequired wells/strips in the MINIGRIP bag along with the dessicant bag and seal tightly.

1. Calibrators
   Dispense 10 µl of each calibrator into the appropriate wells.
2. Samples and controls
   Dispense 10 µl of samples or controls into appropriate wells.
3. Add 200 µl of conjugate (CONJ ENZ) into each well.
4. Incubate for 180 minutes at room temperature (25°C) without shaking.
5. Flick out the contents of the wells over a basin containing bleaching water or aspirate with an automated plate washer.
6. Wash the wells **six** times with an automated system set to 250 µl per well, or by adding 250 µl to each well, flicking out over a basin and blotting the wells on absorbent paper to remove any residual liquid after each washing.
7. Dispense 200 µl of chromogen substrate (SUBS TMB) solution into each well, ensuring that it is initially pale coloured.
8. Incubate for 30 minutes at room temperature (25°C) without shaking.
9. Stop the reaction by adding 50 µl of stop solution (SOLN STOP) to each well.
10. Place the plate on a flat surface, swirl gently to mix contents or use the option « mixing » if your reader has one.

11. Measure the absorbance at 450 nm on a 96 well microplate reader.

***Data Processing***

Draw a calibration curve on semilogarithmic paper by plotting mean absorbance (linear scale) obtained for each calibrator versus its respective concentration expressed in ng/ml (logarithmic scale).
Rat prolactin concentrations in sample may be read directly from the appropriate standard curve.
If a computer is used to calculate the results, the data can be fitted to the appropriate equation :
Non linear regression, Sigmoïdal dose-response or Sigmoïdal dose-response (Variable slope), Polynomial 4$^{th}$ order or spline cubic.

***Example of typical assays***

| | Contents (ng/ml) | ABS 1$^{st}$ Duplicate | ABS 2$^{nd}$ Duplicate | Mean ABS | Abs/ Abs Max % | Rat PRL (ng/ml) |
|---|---|---|---|---|---|---|
| CAL 0 | 0 | 0.085 | 0.088 | 0.087 | 2.76 | |
| CAL 1 | 2.25 | 0.195 | 0.197 | 0.196 | 6.24 | |
| CAL 2 | 4.5 | 0.285 | 0.296 | 0.291 | 9.26 | |
| CAL 3 | 9 | 0.501 | 0.492 | 0.497 | 15.82 | |
| CAL 4 | 18 | 0.861 | 0.869 | 0.865 | 27.54 | |
| CAL 5 | 45 | 1.589 | 1.636 | 1.613 | 51.35 | |
| CAL 6 | 90 | 2.395 | 2.249 | 2.322 | 73.93 | |
| CAL 7 | 180 | 3.090 | 3.192 | 3.141 | 100.00 | |
| Control 1 | | 0.564 | 0.613 | 0.589 | 18.75 | 11.23 |
| Control 2 | | 1.739 | 1.762 | 1.751 | 55.75 | 52.08 |
| Sample 1 | | 0.467 | 0.468 | 0.468 | 14.90 | 8.28 |
| Sample 2 | | 0.905 | 0.916 | 0.911 | 29.00 | 20.00 |
| Sample 3 | | 1.953 | 1.904 | 1.929 | 61.41 | 61.42 |

**Examples of typical assay performed at controlled temperature of 25°C. Do not use for calculations**

**Expected normal values**

It is recommended that each laboratory establishes its own reference values.
Concentrations range for normal male subjects from 6.6 to 24 ng/ml and for normal female subjects from 14 to 24 ng/ml in our preliminary studies.

**Limitation of the procedure**

1. Do not use strongly lipemic, haemolyzed, icteric or turbid specimens
2. Special care is needed to prevent contamination of the substrate by the conjugate. The substrate should be pink, a blue colouration indicates that the reagent has been contaminated and must be discarded. Substrate degradation is increased at temperatures above 25°C.
3. The well washing procedure is critical for the successful performance of the test.
4. The TMB substrate is extremely sensitive to certain handling and storage conditions. Avoid exposure to light, heat and contamination with metal ions or peroxidase.

**Quality control**

Use the controls provided for each assay.

If, in normal using conditions, the controls are out the acceptable ranges, the sample results can't be validated. Please contact the manufacturer.

**Performance characteristics**

***Specificity***

Prolactin from other species can cross react partially in this assay. Cross-reactivity with other rat pituitary hormones measured by radioimmunoassay :

| Compound | Cross-reactivity (%) |
|---|---|
| Rat PRL | 100.0 % |
| Rat TSH | <0.1 % |
| Rat LH | <0.1 % |
| Rat FSH | <0.1 % |
| Rat GH | <0.1 % |
| Mouse PRL | <0.1 % |

***Sensitivity***

**Analytical sensitivity**

The minimum detectable concentration of rat prolactin has been assayed at 0.36 ng/ml and corresponds to the concentration given by two standard deviations above the mean ABS of 25 replicates determinations of the zero standard.

***Imprecision***

| | Repeatability | Within Assay Variation |
|---|---|---|
| | Mean value (ng/ml) | % CV (24 replicates) |
| Sample 1 | 1.1 | 12.56 |
| Sample 2 | 8.63 | 6.67 |
| Sample 3 | 29.48 | 6.64 |
| Sample 4 | 53.86 | 5.97 |

| | Reproducibility | Between Assay Variation |
|---|---|---|
| | Mean value (ng/ml) | % CV (13 replicates) |
| Sample 1 | 2.3 | 17.6 |
| Sample 2 | 7.4 | 9.16 |
| Sample 3 | 17.58 | 9.24 |
| Sample 4 | 56.34 | 11.55 |

***Recovery Test***

When rat prolactin was spiked to rat serum, the recovery of rat prolactin ranges from 79.6 % to 143.8 %

Sample 1:

| **Added Rat PRL (ng/ml)** | **-** | **1.14** | **2.28** | **4.56** | **9.13** | **18.37** | **36.52** | **73.04** |
|---|---|---|---|---|---|---|---|---|
| Assayed Rat PRL (ng/ml) | 4.7 | 6.34 | 7.8 | 10.6 | 14.8 | 26 | 42.5 | 78.2 |
| Rat PRL recovered (ng/ml) | - | 1.64 | 3.1 | 5.9 | 10.1 | 21.3 | 37.8 | 73.5 |
| % Recovery | - | 143.8 | 135.9 | 129.3 | 110.6 | 116.0 | 103.5 | 100.6 |

Sample 2:

| **Added Rat PRL (ng/ml)** | **-** | **1.64** | **3.28** | **13.12** | **26.4** | **52.5** | **105** |
|---|---|---|---|---|---|---|---|
| Assayed Rat PRL (ng/ml) | 0.35 | 1.74 | 2.96 | 10.84 | 22.9 | 48.1 | 93.7 |
| Rat PRL recovered (ng/ml) | - | 1.39 | 2.61 | 10.49 | 22.55 | 47.75 | 93.35 |
| % Recovery | - | 84.8 | 79.6 | 79.9 | 85.4 | 91.0 | 88.9 |

***Linearity: Dilution test***

The dilution test (dilution with CAL 0) indicates that there is immunological identity between the Rat PRL present in serum and the Rat PRL used to calibrate the standard curve.

Sample 1:

| **Dilution factor** | **1** | **2/3** | **1/2** | **1/3** | **1/4** | **1/6** | **1/8** | **1/12** |
|---|---|---|---|---|---|---|---|---|
| Expected Rat PRL (ng/ml) | - | 16.03 | 12.03 | 8.02 | 6.01 | 4.01 | 3.01 | 2.00 |
| Assayed Rat PRL (ng/ml) | 24.05 | 17.22 | 10.29 | 7.53 | 4.82 | 3.59 | 2.11 | 1.61 |
| % Recovery | - | 107.4 | 85.6 | 93.9 | 80.2 | 89.6 | 70.2 | 80.3 |

Sample 2:

| Dilution factor | 1 | 2/3 | 1/2 | 1/3 | 1/4 | 1/6 | 1/8 |
|---|---|---|---|---|---|---|---|
| Expected Rat PRL (ng/ml) | - | 41.13 | 30.85 | 20.57 | 15.43 | 10.28 | 7.71 |
| Assayed Rat PRL (ng/ml) | 61.7 | 38.10 | 30.05 | 17.50 | 12.90 | 8.40 | 6.10 |
| % Recovery | - | 92.6 | 97.4 | 85.1 | 83.6 | 81.7 | 79.1 |

**Bibliography**

1. D. DOMBROWICZ, B. SENTE, J. CLOSSET, G.HENNEN Dose-dependent effects of human prolactin on immature hypox rat testis.
   Endocrinology, 130 : 695-700, 1992.
2. E. REITER, P. BONNET, B. SENTE, D. DOMBROWICZ, J. de LEVAL, J. CLOSSET, G. HENNEN Growth hormone and prolactin stimulate androgen receptor, insulin-like growth factor-I (IGF-1) and IGF-I receptor levels in the prostate of immature rats.
   Mol. Cell. Endocrinol., 88 : 77-87, 1992.
3. E. REITER, S. LARDINOIS, M. KLUG, B. SENTE, B. HENNUY, J. CLOSSET, G. HENNEN Androgen-independent effects of prolactin on the different lobes of the immature rat prostate.
   Mol. Cell. Endocrinol., 112 : 113-122, 1995.

## Symbols used with DRG Assays

| Symbol | English | Deutsch | Français | Español | Italiano |
|---|---|---|---|---|---|
|  | Consult instructions for use | Gebrauchsanweisung beachten | Consulter les instructions d'utilisation | Consulte las instrucciones de uso | Consultare le istruzioni per l'uso |
| CE | European Conformity | CE-Konfirmitäts-kennzeichnung | Conformité aux normes européennes | Conformidad europea | Conformità europea |
| IVD | In vitro diagnostic device | In-vitro-Diagnostikum | Usage Diagnostic in vitro | Para uso Diagnóstico in vitro | Per uso Diagnostica in vitro |
| RUO | For research use only | Nur für Forschungszwecke | Seulement dans le cadre de recherches | Sólo para uso en investigación | Solo a scopo di ricerca |
| REF | Catalogue number | Katalog-Nr. | Numéro de catalogue | Número de catálogo | Numero di Catalogo |
| LOT | Lot. No. / Batch code | Chargen-Nr. | Numéro de lot | Número de lote | Numero di lotto |
|  | Contains sufficient for <n> tests/ | Ausreichend für "n" Ansätze | Contenu suffisant pour "n" tests | Contenido suficiente para <n> ensayos | Contenuto sufficiente per "n" saggi |
|  | Storage Temperature | Lagerungstemperatur | Température de conservation | Temperatura de conservación | Temperatura di conservazione |
|  | Expiration Date | Mindesthaltbarkeits-datum | Date limite d'utilisation | Fecha de caducidad | Data di scadenza |
|  | Legal Manufacturer | Hersteller | Fabricant | Fabricante | Fabbricante |
| Distributed by | Distributor | Vertreiber | Distributeur | Distribuidor | Distributore |
| Content | Content | Inhalt | Conditionnement | Contenido | Contenuto |
| Volume/No. | Volume / No. | Volumen/Anzahl | Volume/Quantité | Volumen/Número | Volume/Quantità |