

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 of a 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 kit 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 desiccant 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) H₂SO₄ 2M.

4.8. BUF WASH 20x

1 bottle (60 ml) concentrated buffer solution containing preservative (Proclin 0.05%). Pour 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.

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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, Sigmoidal dose-response or Sigmoidal dose-response (Variable slope), Polynomial 4th 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.

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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

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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:

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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.

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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
	European Conformity	CE-Konformitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	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	Mindesthaltbarkeitsdatum	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