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Human Prolactin/PRL SimpleStep ELISA® Kit

For the quantitative measurement of Prolactin/PRL in Human serum, plasma, milk, and cell culture supernatant.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Prolactin/PRL *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Prolactin/PRL protein in Human serum, plasma, milk, and cell culture supernatant.

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB Development Solution is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

Prolactin is a secreted neuroendocrine pituitary hormone. Prolactin acts primarily on the mammary gland by promoting lactation, but has also additional pleiotropic effects both in males and females. Mature prolactin is a 199 amino acid long secreted monomeric protein. Prolactin can be also found in less biologically active form, macroprolactin, in a complex with IgG.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed



Add 50 µL standard or sample to appropriate wells



Add 50 µL Antibody Cocktail to all wells



Incubate at room temperature for 1 hour



Aspirate and wash each well three times with 350 µL 1X Wash Buffer PT



Add 100 µL TMB Development Solution to each well and incubate for 10 minutes.



Add 100 µL Stop Solution and read OD at 450 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

4. Storage and Stability

Store kit at +4°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Human Prolactin/PRL Capture Antibody 10X	600 µL	+4°C
Human Prolactin/PRL Detector Antibody 10X	600 µL	+4°C
Human Prolactin/PRL Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 5BR	6 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
TMB Development Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
Sample Diluent 25BS	20 mL	+4°C
Sample Diluent NS*	12 mL	+4°C
Anti-tag coated microplate (12 x 8 well strips)	96 Wells	+4°C
Plate Seal	1	+4°C

*Sample Diluent NS is provided but not necessary for this product.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.

- **To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.**
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.
- Sample Diluent 25BS may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 1X Wash Buffer PT:

Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

9.2 Antibody Cocktail:

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 5BR. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 5BR. Mix thoroughly and gently.

10. Standard Preparation

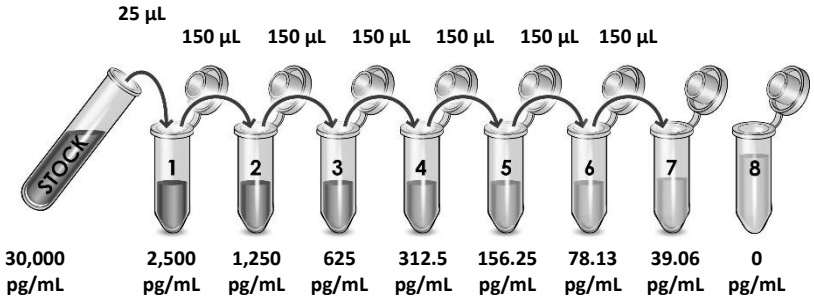
- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the Prolactin/PRL by adding that volume of Sample Diluent 25BS indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the Prolactin/PRL standard by adding 500 µL Sample Diluent 25BS. Hold at room temperature for 10 minutes and mix gently. This is the 30,000 pg/mL **Stock Standard** Solution.

10.2 Label eight tubes, Standards 1– 8.

10.3 Add 275 µL Sample Diluent 25BS into tube number 1 and 150 µL of Sample Diluent 25BS into numbers 2-8.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



11. Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Plasma - Heparin	25 – 100%
Plasma - EDTA	12.5 – 100%
Plasma - Citrate	12.5 – 100%
Serum	1.56 – 12.5%
Pregnant Serum	0.05 – 0.78%
Milk	3.12 – 12.5%
Cell Culture Supernatant	3.13 – 50%

11.1 Plasma:

Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples into Sample Diluent 25BS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.2 Serum:

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples into Sample Diluent 25BS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

11.3 Cell Culture Supernatants:

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Or dilute samples into Sample Diluent 25BS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

11.4 Milk:

De-fat milk samples as follows. Centrifuge milk samples at 500 x g for 15 minutes at 4ºC and collect the aqueous fraction using syringe attached to needle. Centrifuge the aqueous fraction at 3,000 x g for 15 minutes at 4ºC and collect the final aqueous fraction (de-fatted milk) using syringe attached to needle. Dilute the de-fatted milk samples at least 1:8 in Sample Diluent 25BS and assay. Store un-diluted de-fatted milk at -20ºC or below. Avoid repeated freeze-thaw cycles.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or "edge effects" have not been observed with this assay.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.
Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.

13.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

13.9 Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed

time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Δ **Note**: that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

13.10 Analyze the data as described below.

14. Calculations

14.1 Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

14.2 **Create a standard curve** by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

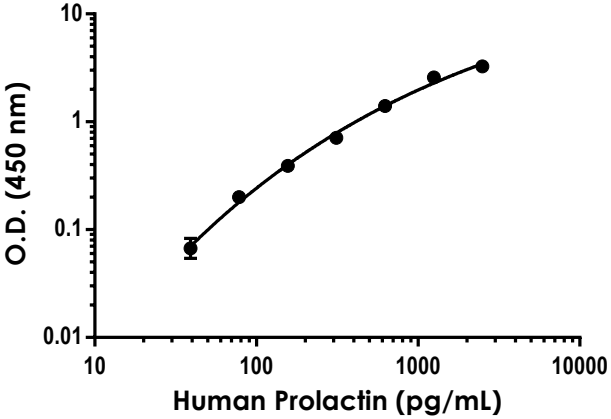
Δ **Note:** Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.3 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted **absorbance values against the standard curve**. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

14.4 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements			
Concentration (pg/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.145	0.151	0.148
39.06	0.226	0.21	0.216
78.13	0.346	0.349	0.348
156.25	0.527	0.548	0.537
312.5	0.891	0.825	0.858
625	1.569	1.53	1.549
1,250	2.759	2.685	2.722
2,500	3.419	3.39	3.404

Figure 1. Example of human Prolactin/PRL standard curve in Sample Diluent 25BS. The Prolactin/PRL standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. Calibration

This immunoassay is calibrated against a highly purified human Prolactin/PRL protein.

17. Typical Sample Values

SENSITIVITY –

The calculated minimal detectable dose (MDD) is 17.64 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=8) and adding 2 standard deviations then extrapolating the corresponding concentration.

RECOVERY –

Three concentrations of Prolactin/PRL protein were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
12.5% Plasma – Heparin	90	88 – 91
12.5% Plasma – Citrate	76	70 – 82
12.5% Plasma – EDTA	79	76 – 82
6.25% Serum	89	86 – 91
6.25% Milk	106	100 – 109
25% (RPMI Media + 10% FBS)	114	108 – 118

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native Prolactin/PRL was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent 25BS.

Dilution Factor	Interpolated value	12.5% Human Serum	100% Human Plasma (Citrate)	100% Human Plasma (EDTA)	100% Human Plasma (Heparin)
Undiluted	pg/mL	139	449	698	818
	% Expected value	100	100	100	100
2	pg/mL	69	263	370	430
	% Expected value	100	117	106	105
4	pg/mL	28	127	190	182
	% Expected value	81	113	109	89
8	pg/mL	NL	55	81	NL
	% Expected value		97	92	

NL – Non-Linear

Native Prolactin/PRL was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent 25BS.

Dilution Factor	Interpolated value	0.78% Pregnant Human Serum	12.5% Human Milk
Undiluted	pg/mL	355	165
	% Expected value	100	100
2	pg/mL	176	75
	% Expected value	99	92
4	pg/mL	72	35
	% Expected value	81	85
8	pg/mL	47	NL
	% Expected value	105	

NL – Non-Linear

Recombinant Prolactin/PRL was spiked into the following biological samples and diluted in a 2-fold dilution series in Sample Diluent 25BS.

Dilution Factor	Interpolated value	50% RPMI Media + 10% FBS
Undiluted	pg/mL	13,338
	% Expected value	100
2	pg/mL	645
	% Expected value	96
4	pg/mL	312
	% Expected value	93
8	pg/mL	178
	% Expected value	106
16	pg/mL	70
	% Expected value	84

PRECISION –

Mean coefficient of variations of interpolated values of Prolactin/PRL from three concentrations of serum within the working range of the assay.

	Intra-Assay	**Inter-Assay**
n =	9	3
CV (%)	14	12.6

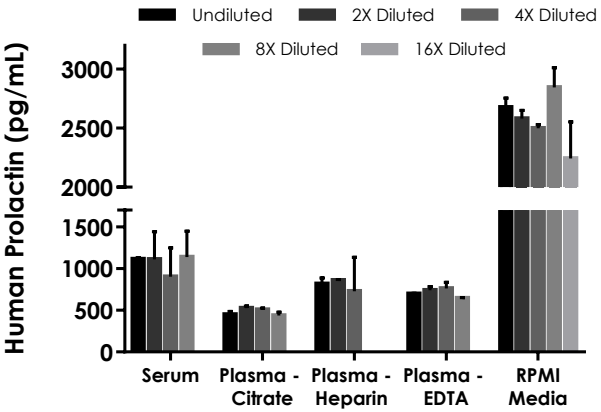


Figure 2. Interpolated concentrations of native Prolactin/PRL in human serum and plasma and of spiked Prolactin in cell culture media samples. The concentrations of Prolactin/PRL were measured in duplicates, interpolated from the Prolactin/PRL standard curves and corrected for sample dilution. Undiluted samples are as follows: serum 12.5%, plasma (citrate) 100%, plasma (heparin) 100%, plasma (EDTA) 100%, and RPMI media 50%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Prolactin/PRL concentration was determined to be 1,066 pg/mL in serum, 480 pg/mL in plasma (citrate), 801 pg/mL in plasma (heparin), and 711 pg/mL in plasma (EDTA).

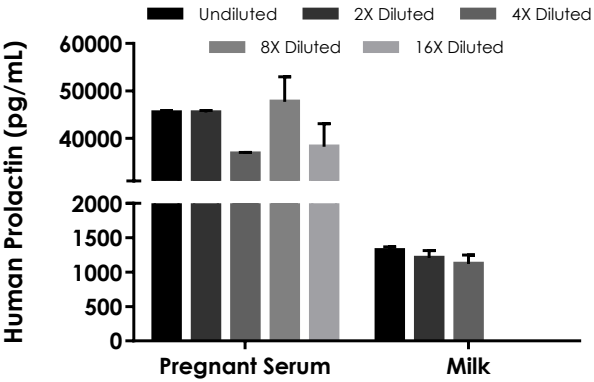


Figure 3. Interpolated concentrations of native Prolactin/PRL in human pregnant serum and milk. The concentrations of Prolactin/PRL were measured in duplicate and interpolated from the Prolactin/PRL standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Prolactin/PRL concentration was determined to be 42,647 pg/mL in pregnant serum and 1,214 pg/mL in milk.

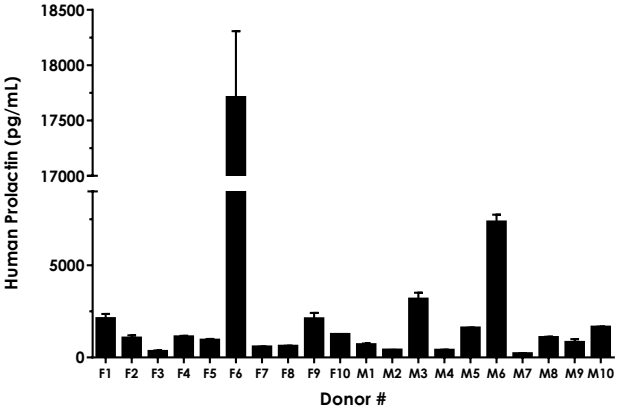


Figure 4. Serum from ten individual healthy human female donors and ten individual healthy male donors was measured in duplicate. Interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Prolactin/PRL concentration in females was determined to be 2,778 pg/mL with a range of 294 – 18,132 pg/mL. The mean Prolactin concentration in males was determined to be 1,733 pg/mL with a range of 179 – 7,626 pg/mL.

18. Assay Specificity

This kit recognizes both native and recombinant Human Prolactin/PRL protein in serum, plasma, milk, and cell culture supernatant samples only.

Cell and tissue extract samples have not been tested with this kit.

CROSS REACTIVITY

Recombinant mouse Prolactin was prepared at 125 ng/mL and 2.5 ng/mL and assayed for cross reactivity. No cross-reactivity was observed.

INTERFERENCE

Recombinant human Prolactin Receptor was prepared at 125 ng/mL and 2.5 ng/mL and tested for interference. No interference with was observed.

19. Species Reactivity

This kit recognizes Human Prolactin/PRL protein.

Other species reactivity was determined by measuring 100% serum samples of various species, interpolating the protein concentrations from the Human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in Human serum assayed at the same dilution.

Reactivity < 3% was determined for the following species:

- Mouse
- Rat
- Cow

Please contact our Technical Support team for more information.

20. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB Development Solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.

21. Notes

Technical Support

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