DRG® Leptin Receptor (human) (EIA-3527)

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

This kit is intended for Research Use Only. Not for use in diagnostic procedures.

INTENDED USE

The Human Leptin Receptor ELISA is a sandwich enzyme immunoassay for measurement of human leptin receptor.

Features

- The total assay time is less than 2.5 hours.
- The kit measures leptin receptor in serum, plasma (EDTA, citrate, heparin) and tissue culture medium.
- Assay format is 96 wells.
- Quality Controls are human serum based.
- Standard is recombinant protein based.
- Components of the kit are provided ready to use, concentrated or lyophilized.

STORAGE, EXPIRATION

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.

TEST PRINCIPLE

In this Human Leptin Receptor ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with monoclonal anti-human leptin receptor antibody. After 60 minutes incubation and washing, monoclonal anti-human leptin receptor antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with captured leptin receptor. Following another washing step, the remaining HRP conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured spectrophotometrically at 450 nm. The absorbance is proportional to the concentration of leptin receptor. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

PRECAUTIONS

- **For professional use only.**
- Wear gloves and laboratory coats when handling materials.

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- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.
- This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents.
- This kit contains components of animal origin. However, these materials should be handled as potentially infectious.
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary.
- The materials must not be pipetted by mouth.

TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed.
- Use thoroughly clean glassware.
- Use deionized (distilled) water, stored in clean containers.
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent.
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light.
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements.

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REAGENT SUPPLIED

Kit Components	State	Quantity
Antibody Coated Microtiter Strips	ready to use	96 wells
Conjugate Solution	ready to use	13 ml
Human Leptin Receptor Standards (2, 5, 10, 20, 50, 100 ng/ml)	liquid	6 x 0.35 ml
Quality Control High	lyophilized	1 vial
Quality Control Low	lyophilized	1 vial
Dilution Buffer	ready to use	13 ml
Wash Solution Concentrate (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml
Product Data Sheet + Certificate of Analysis		1 pc

MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 10-1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter
- Software package facilitating data generation and analysis (optional)

PREPARATION OF REAGENTS

All reagents need to be brought to room temperature prior to use.
Always prepare only the appropriate quantity of reagents for your test.
Do not use components after the expiration date marked on their label.

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Assay reagents supplied ready to use

Antibody Coated Microtiter Strips
Stability and storage:
Return the unused strips to the provided aluminium zip-sealed bag with desiccant and seal carefully.
Remaining Microtiter Strips are stable 3 month when stored at 2-8°C and protected from the moisture.

Conjugate Solution

Dilution Buffer

Substrate Solution

Stop Solution

Stability and storage:
Opened reagents are stable 3 month when stored at 2-8°C.

Assay reagents supplied concentrated or lyophilized

Human Leptin Receptor Standards
Dilute each concentration of standard 3x with the Dilution Buffer prior to the assay,
e.g. 50 µl of standard + 100 µl of Dilution Buffer for singlets,
or preferably 100 µl of standard + 200 µl of Dilution Buffer for duplicates. Mix well (not to foam).
Stability and storage:
Opened standards are stable 3 month when stored at 2-8°C. **Do not store the diluted (3x) standards.**

Quality Controls High, Low
Refer to the Certificate of Analysis for current Quality Control concentrations!!!
Reconstitute each Quality Control (High and Low**) with 350 µl of distilled (deionized) water** just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).
Dilute reconstituted Quality Controls **3x with Dilution Buffer**,
e.g. 50 µl of Quality Control + 100 µl of Dilution Buffer when assaying samples in singlets,
or preferably 100 µl of Quality Control + 200 µl of Dilution Buffer for duplicates. **Mix well** (not to foam).
Stability and storage:
The reconstituted Quality Controls must be used immediately or aliquoted and frozen at -20°C for 3 month.
Avoid repeated freeze/thaw cycles. **Do not store the diluted Quality Controls.**

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Wash Solution Concentrate (10x)
Dilute Wash Solution Concentrate (10x) ten fold in distilled water to prepare a 1x working solution.
Example: 100 ml of Wash Solution Concentrate (10x)+ 900 ml of distilled water for use of all 96-wells.
Stability and storage:
The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

PREPARATION OF SAMPLES

The kit measures leptin receptor in serum or plasma (EDTA, citrate, heparin) and tissue culture medium. Samples should be assayed immediately after collection or should be stored at -20°C. Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Dilute samples 3x with Dilution Buffer just prior to the assay
e.g. 50 µl of sample + 100 µl of Dilution Buffer for singlets,
or preferably 100 µl of sample + 200 µl of Dilution Buffer for duplicates.
Mix well (not to foam). Vortex is recommended.

Stability and storage:

Samples should be stored at -20°, or preferably at -70°C for long-term storage. Avoid repeated freeze/ thaw cycles.
Do not store the diluted samples.

See Chapter 13 for stability of serum and plasma samples when stored at 2-8°C, effect of freezing/thawing and effect of sample matrix (serum/plasma) on the concentration of leptin receptor.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

Ask for protocol at DRG if assaying other samples.

ASSAY PROCEDURE

1. Pipet **100 µl** of diluted Standards, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells. See Figure 1 for example of work sheet
2. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells **5-times** with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.

4. Add **100 µl** of Conjugate Solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells **5-times** with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add **100 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
8. Incubate the plate for **10 minutes** at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C. Do not shake the plate during the incubation.
9. Stop the colour development by adding **100 µl** of Stop Solution.
10. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 – 650 nm). Substract readings at 630 nm (550 – 650 nm) fro the reading at 450 nm.
    The absorbance should be read within 5 minutes following step 9.

Note:
If the microplate reader is not capable of reading absorbance greater than the absorbance of the highest standard, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine leptin receptor concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.
Note 2:
Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	**Standard 100**	Blank	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 50**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 20**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 10**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 5**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 2**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	**QC High**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	**QC Low**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

Figure 1: Example of a work sheet.

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CALCULATIONS

Most microplate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the mean absorbance at 450 nm (Y) of Standards against log of the known concentration (X) of Standards, using the four-parameter algorithm. Results are reported as concentration of leptin receptor (ng/ml) in samples.

Alternatively, the logit log function can be used to linearize the standard curve (i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of Standards.

Samples, Quality Controls and Standards are all diluted 3x prior to analysis, so there is not need to take this dilution factor into account.

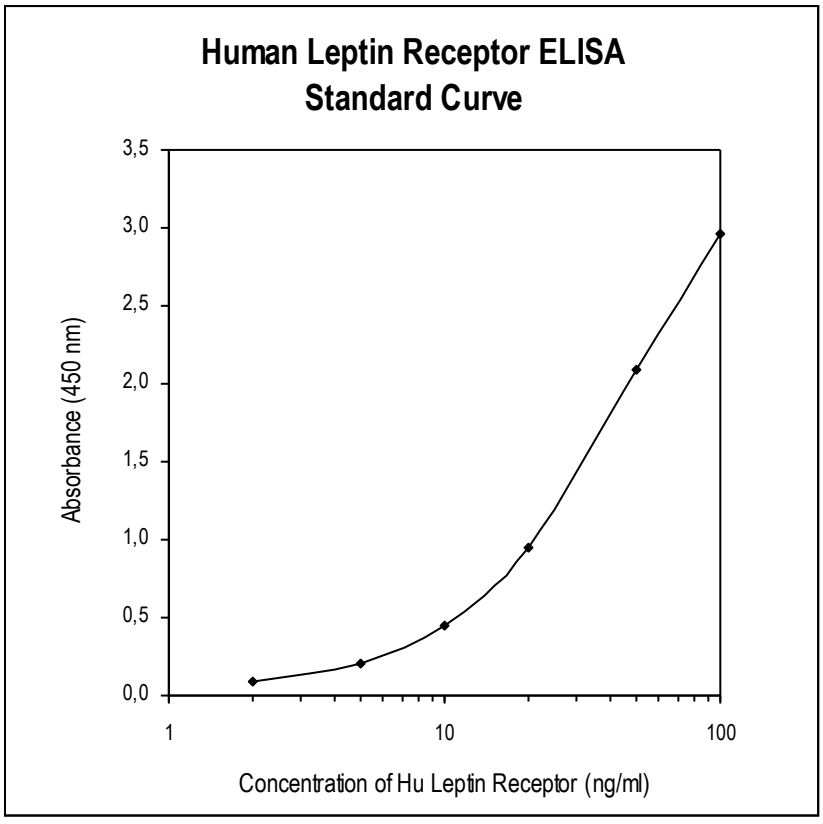

Figure 2: Typical Standard Curve for Human Leptin Receptor ELISA.

Stability of samples stored at 2-8°C

Samples should be stored at –20°C. However, no decline in concentration of leptin receptor was observed in serum and plasma samples after 10 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and sodium azide, resulting in the final concentration of 0.03% and 0.1%, respectively.

Sample	Incubation Temp, Period	Serum *(ng/ml)*	Plasma *(ng/ml)*		
			EDTA	Citrate	Heparin
1	-20°C	49.75	41.48	38.82	35.69
	2-8°C, 1 day	47.13	43.03	41.38	41.19
	2-8°C, 10 days	45.04	44.28	46.02	40.49
2	-20°C	22.36	23.27	21.70	22.97
	2-8°C, 1 day	21.79	24.54	21.81	24.10
	2-8°C, 10 days	23.56	22.69	22.93	19.35
3	-20°C	33.28	33.34	35.09	34.46
	2-8°C, 1 day	35.80	33.49	30.82	32.29
	2-8°C, 10 days	35.15	33.96	31.77	35.45

Effect of Freezing/Thawing

No decline was observed in concentration of human leptin receptor in serum and plasma samples after repeated (5x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

Sample	Number of f/t cycles	Serum *(ng/ml)*	Plasma *(ng/ml)*		
			EDTA	Citrate	Heparin
1	1x	21.39	20.89	16.77	21.79
	3x	18.69	18.31	16.20	22.72
	5x	20.06	20.47	16.43	21.15
2	1x	26.89	24.71	23.33	27.14
	3x	26.77	26.91	22.29	26.58
	5x	25.24	24.80	20.93	24.36
3	1x	18.07	18.51	16.00	18.96
	3x	17.57	17.95	17.19	19.73
	5x	19.39	18.55	17.29	19.62

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Reference ranges

It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological reference ranges for leptin receptor levels with the assay.

DEFINITION OF THE CALIBRATOR

The Standard used in this kit is recombinant human IgG-Fc-fragment - human OB-R dimeric chimera, which is different from the native soluble OB-R that is measured in human serum. Mature OB-R is a disulfide-linked homodimeric protein. As a result of glycosylation, recombinant human leptin receptor/Fc chimera migrates as a 205 kDa protein in SDS-PAGE and we used to employ the unit U/ml.
From the lot RD-877 we started using the unit ng/ml.
1 ng OB-R/ml = 1 U OB-R/ml (previously used).
It can be to recalculate previous results with factor 1.0; e.g. concentration of leptin receptor 24.50 U/ml in a sample measured in previous assays corresponds to 24.50 ng/ml of leptin receptor measured in this assay.

METHOD COMPARISON

The Human Leptin Receptor ELISA has not been compared to any other immunoassay.

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TROUBLESHOOTING AND FAQS

Weak signal in all wells

Possible explanations:

- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing
- Improper mixing Standards, Quality Controls or samples

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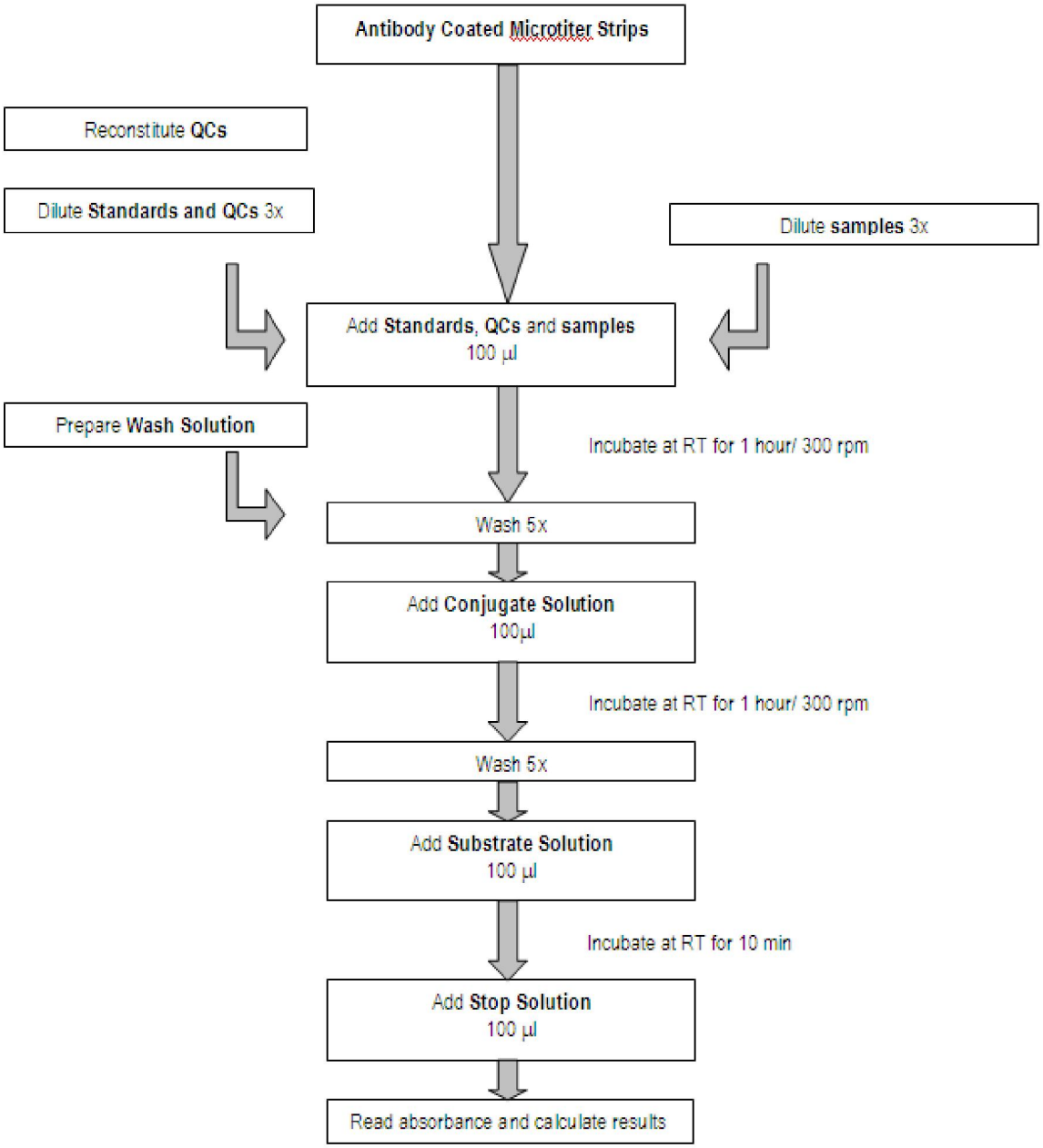
Assay Procedure Summary



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