Murine IL-15/IL-15R Complex ELISA Kit

Instructions for use

Catalogue numbers: 1x96 tests: 660.090.096

For research use only

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Murine IL-15/IL-15R Complex ELISA KIT

1. Intended use

The Murine IL-15/IL-15R Complex ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of Murine IL-15/IL-15R Complex. **The Murine IL-15/IL-15R Complex ELISA is for research use only. Not for diagnostic or therapeutic procedures.**

2. Introduction

2.1. Summary

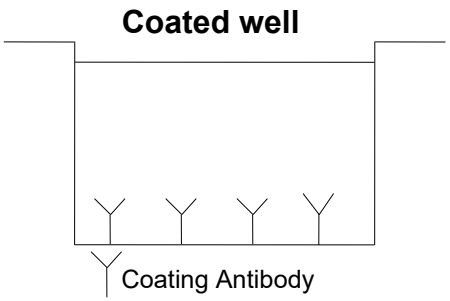
Interleukin-15 (IL-15) is a 14 kDa proinflammatory protein that has been shown to play a role in the activation of neutrophils, dendritic cells, and macrophages, and is essential to the development and survival of NK cells and CD8 T-cells. IL-15 activates Jak/Stat pathways by signaling through a heterotrimeric receptor. Two of the subunits of this receptor, β and γ, are shared with the IL-2 receptor, while the α subunit is unique to IL-15.

Despite the expression of IL-15 mRNA in many cell types, the secreted protein is rarely detectable in biological samples. Recent research suggests that IL-15 is retained inside the cell and is only secreted in complex with its unique receptor, IL-15Rα. This chaperoning is required from the ER to Golgi through to secretion. The cytokine/receptor complex may stay bound to the cell surface, where it can be trans-presented to cells expressing the βγ subunits of the receptor, or may be secreted in a soluble form that lacks the transmembrane domain of the receptor. The soluble IL-15/IL-15R complex is produced by murine dendritic cells, fibroblasts, and macrophages, and demonstrates a 10-100-fold increase in agonistic activity over IL-15 alone.

2.2. Principle of the method

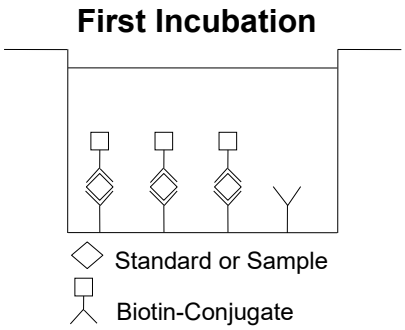
An anti-Murine IL-15/IL-15R Complex coating antibody is adsorbed onto microwells.

Figure 1



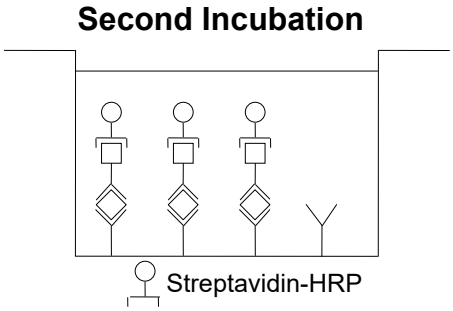
Murine IL-15/IL-15R Complex present in the sample or standard binds to antibodies adsorbed to the microwells and a biotin-conjugated anti-Murine IL-15/IL-15R Complex antibody is added and binds to Murine IL-15/IL-15R Complex captured by the first antibody.

Figure 2



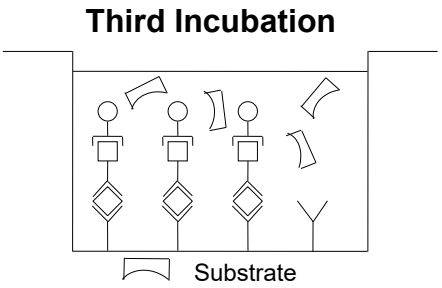
Following incubation unbound biotin-conjugated anti-Murine IL-15/IL-15R Complex antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-Murine IL-15/IL-15R Complex antibody.

Figure 3



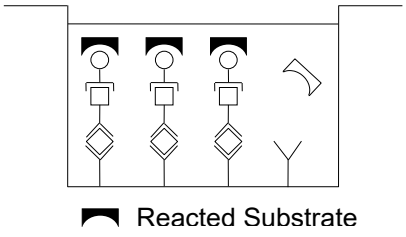
Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

Figure 4



A coloured product is formed in proportion to the amount of Murine IL-15/IL-15R Complex present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 Murine IL-15/IL-15R Complex standard dilutions and Murine IL-15/IL-15R Complex sample concentration determined.

Figure 5



3. Reagents provided and reconstitution

REAGENTS (store at 2-8°C)	Quantity 1x96 well kit Cat n°660.090.096	RECONSTITUTION
96-wells precoated microtiter plate	1	Ready-to-use
Plate covers	2	-
Biotin-Conjugate anti-mIL-15	1 vial (70 µl)	Dilute in Assay Diluent 1X (see Assay preparation, section 8)
Streptavidin-HRP	1 vial (150 µl)	Dilute in Assay Diluent 1X (see Assay preparation, section 8)
mIL-15 Standard: 400 pg/ml	1 vial	Reconstitute as directed on the vial (see Assay preparation, section 8)
Assay Buffer Concentrate	1 vial (5ml)	20X concentrate. Dilute in distilled water
Wash Buffer Concentrate	1 vial (50 ml)	20X concentrate. Dilute in distilled water
Sample Diluent	1 vial (12 ml)	Ready -to-use
Calibrator Diluent	1 vial (5 ml)	Ready -to-use
Substrate Solution (TMB)	1 vial (15 ml)	Ready-to-use
Stop Solution (1M Phosphoric Acid)	1 vial (15 ml)	Ready-to-use

4. Materials required but not provided

- Microtiter plate reader fitted with appropriate filters (450nm required with optional 620nm reference filter)
- Microplate washer or wash bottle
- 10, 50, 100, 200 and 1,000µl adjustable single channel micropipettes with disposable tips
- 50-300µl multi-channel micropipette with disposable tips
- Multichannel micropipette reagent reservoirs
- Distilled water
- Vortex mixer
- Miscellaneous laboratory plastic and/or glass, if possible sterile

5. Storage Instructions

Store kit reagents between 2° and 8°C. Immediately after use remaining reagents should be returned to cold storage (2° to 8°C). Expiry of the kit and reagents is stated on labels.

Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

6. Specimen collection, processing & storage

Cell culture supernatant, serum and plasma (EDTA) were tested with this assay. Other biological fluids might be suitable for use in the assay. Remove serum and plasma from the clot as soon as possible after clotting and separation.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Pay attention to a possible **"Hook Effect"** due to high sample concentrations.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive murine IL-15. If samples are to be run within 24 hours, they may be stored at 2° to 8°C.
Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Do not thaw samples in a 37°C water bath. Do not vortex or sharply agitate samples.

7. Safety & precautions for use

- Handling of reagents, serum or plasma specimens should be in accordance with local safety procedures, e.g. CDC/NIH Health manual: "Biosafety in Microbiological and Biomedical Laboratories" 1984.
- Laboratory gloves should be worn at all times.
- Avoid any skin contact with Stop solution and TMB. In case of contact, wash thoroughly with water.
- Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
- Do not pipette by mouth.
- When not in use, kit components should be stored refrigerated as indicated on vials or bottles labels.
- All reagents should be warmed to room temperature before use. Lyophilized standards should be discarded after use.
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from deterioration.
- Cover or cap all reagents when not in use.
- Do not mix or interchange reagents between different lots.
- Do not use reagents beyond the expiration date of the kit.
- Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross contamination, for the dispensing of Stop solution and substrate solution, avoid pipettes with metal parts.
- Use a clean plastic container to prepare the washing solution.
- Thoroughly mix the reagents and samples before use by agitation or swirling.
- All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
- The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent colour development. Warning TMB is toxic avoid direct contact with hands. Dispose off properly.
- If a dark blue colour develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbances within 1 hour after completion of the assay.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
- Follow incubation times described in the assay procedure.
- Dispense the TMB solution within 15 min of the washing of the microtiter plate.

8. Assay Preparation

Bring all reagents to room temperature before use

8.1. Assay Design

Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running zeros and standards. Each sample, standard and zero should be tested **in duplicate**. Remove sufficient Microwell Strips for testing from the pouch immediately prior to use. Return any wells not required for this assay with desiccant to the pouch. Seal tightly and return to 2-8ºC storage.

Example plate layout (example shown for a 7 point standard curve)

	Standards		Sample Wells									
	1	2	3	4	5	6	7	8	9	10	11	12
A	400	400										
B	200	200										
C	100	100										
D	50	50										
E	25	25										
F	12.5	12.5										
G	Zero	Zero										
H												

All remaining empty wells can be used to test samples in duplicate

8.2. Preparation of Wash Buffer

Pour entire contents (50 ml) of the Wash Buffer Concentrate (20x) into a clean 1000 ml graduated cylinder. Bring to final volume of 1000 ml with glass-distilled or deionized water.
Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.

Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20x) (ml)	Distilled Water (ml)
1 - 6	25	475
1 - 12	50	950

8.3. Preparation of Assay Buffer

Pour the entire contents (5 ml) of the **Assay Buffer Concentrate** (20x) into a clean 100 ml graduated cylinder. Bring to final volume of 100 ml with distilled water. Mix gently to avoid foaming.

Store at 2° to 8°C. Please note that the Assay Buffer (1x) is stable for 30 days.

Assay Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (20x) (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

8.4. Preparation of Standard

Reconstitute **Murine IL-15/IL-15R Complex standard** by addition of Calibrator diluent.

Reconstitution volume is stated on the label of the standard vial. Allow the reconstituted standard to sit for 10-30 minutes. Swirl or mix gently to insure complete and homogeneous solubilisation.
(concentration of reconstituted standard = 400 pg/ml).

The standard has to be used immediately after reconstitution and cannot be stored.

Label 6 tubes, one for each standard point. S2, S3, S4, S5, S6, S7.
The reconstituted standard serves as S1. Then prepare 1:2 serial dilutions for the standard curve as follows: Pipette 150 µl of Calibrator Diluent into each tube S2-S7. Pipette 150 µl of reconstituted standard (concentration = 400 pg/ml) into the first tube, labelled S2, and mix (concentration of standard 2 = 200 pg/ml). Pipette 150 µl of this dilution into the second tube, labelled S3, and mix thoroughly before the next transfer. Repeat serial dilutions 4 more times thus creating the points of the standard curve ranging from 400 to 6.25 pg/ml.

Calibrator diluent serves as blank.

8.5. Preparation of Biotin-Conjugate

Please note that the Biotin-Conjugate should be used within 30 minutes after dilution.

Make a 1:100 dilution of the concentrated **Biotin-Conjugate** solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (ml)	Assay Buffer (1x) (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

8.6. Preparation of Streptavidin-HRP

Please note that the Streptavidin-HRP should be used within 30 minutes after dilution.

Make a 1:200 dilution of the concentrated **Streptavidin-HRP** solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Streptavidin-HRP (ml)	Assay Buffer (1x) (ml)
1 - 6	0.03	5.97
1 - 12	0.06	11.94

9. Method

We strongly recommend that every vial is mixed thoroughly without foaming prior to use except the standard vial which must be mixed gently by inversion only.

Prepare all reagents as shown in section 8.
Note: Final preparation of Biotin conjugate (section 8.5) and Streptavidin-HRP (section 8.6) should occur immediately before use.

Assay Step		Details
1.	Wash	a) Dispense 0.4 ml of **1x washing solution** into each well b) Aspirate the contents of each well c) Repeat steps a and b Do not allow wells to dry before use
2.	Addition	Add 50µl of **Sample diluent** to all wells
3.	Preparation	**Prepare Standard curve** as shown in section 8.4.
4.	Addition	Add 50µl of each **Standard, Sample and zero (calibrator diluent)** in duplicate to appropriate number of wells
5.	Addition	Add 50µl of diluted **biotin-conjugate** to all wells
6.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **2 hours** if available on a microplate shaker
7.	Wash	Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well b) Dispense 0.4 ml of **1x washing solution** into each well c) Aspirate the contents of each well d) Repeat step b and c another three times
8.	Addition	Add 100µl of **Streptavidin-HRP** solution into all wells
9.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **1 hour** if available on a microplate shaker
10.	Wash	Repeat wash step 7.
11.	Addition	Add 100µl of ready-to-use **TMB Substrate Solution** into all wells
12.	Incubation	Incubate in the dark for **30 minutes*** at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.
13.	Addition	Add 100µl of **Stop Reagent** into all wells
Read the absorbance value of each well (immediately after step 13.) on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm as the reference wave length (610 nm to 650 nm is acceptable).		

**Incubation time of the substrate solution is usually determined by the ELISA reader performance. Many ELISA readers only record absorbance up to 2.0 O.D. Therefore the colour development within individual microwells must be observed by the analyst, and the substrate reaction stopped before positive wells are no longer within recordable range.*

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

10. Data Analysis

Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean value.

Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the Murine IL-15/IL-15R Complex concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).

To determine the concentration of circulating Murine IL-15/IL-15R Complex for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding Murine IL-15/IL-15R Complex concentration.

If instructions in this protocol have been followed samples have not been diluted, the concentration read from the standard curve must not be multiplied by the dilution factor.

Calculation of samples with a concentration exceeding standard 1 may will result in incorrect, low Murine IL-15/IL-15R Complex levels (Hook Effect). Such samples require further external predilution according to expected Murine IL-15/IL-15R Complex values with Sample Diluent in order to precisely quantitate the actual Murine IL-15/IL-15R Complex level.

It is suggested that each testing facility establishes a control sample of known Murine IL-15/IL-15R Complex concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.

Figure 6
Representative standard curve for Murine IL-15/IL-15R Complex ELISA. Murine IL-15/IL-15R Complex was diluted in serial 2-fold steps in Calibrator Diluent. Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

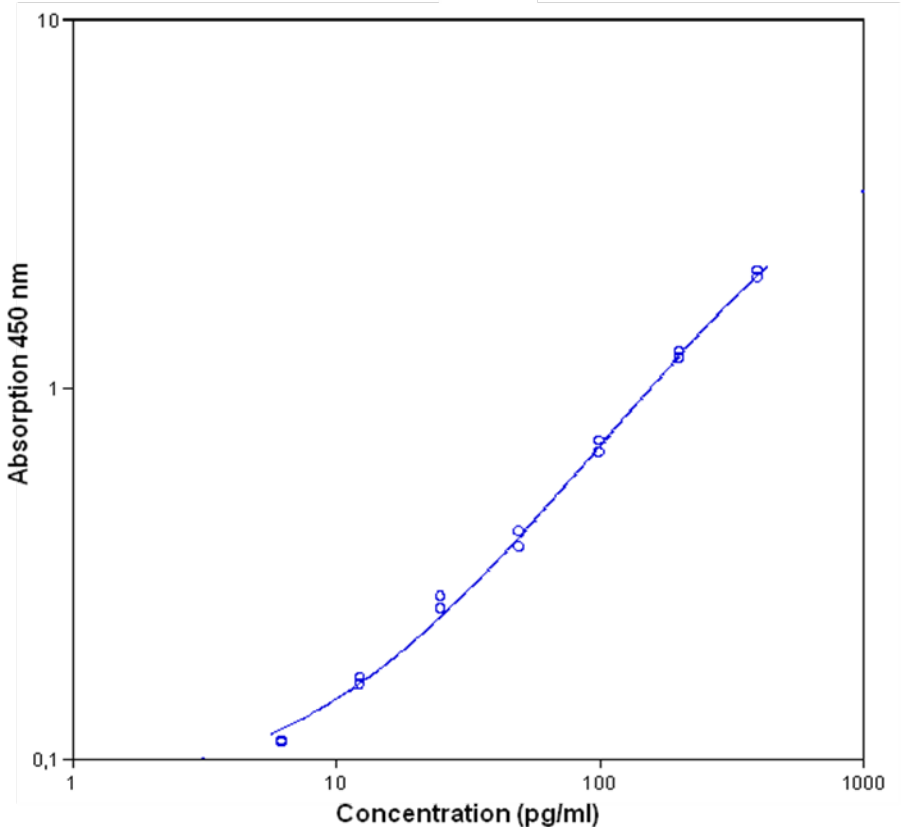


Table 1
Typical data using the Murine IL-15/IL-15R Complex ELISA
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	Murine IL-15/IL-15R Complex Concentration (pg/ml)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	400.0	2.086 2.087	2.086	0.0
2	200.0	1.208 1.258	1.233	2.0
3	100.0	0.668 0.720	0.694	3.8
4	50.0	0.371 0.411	0.391	5.1
5	25.0	0.272 0.254	0.263	3.3
6	12.5	0.158 0.165	0.162	2.0
Blank	0.0	0.051 0.052	0.051	0.8

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus colour intensity. Values measured are still valid.

11. Assay limitations

Do not extrapolate the standard curve beyond the maximum standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples above the maximum standard concentration must be diluted with Standard diluent or with your own sample buffer to produce an OD value within the range of the standard curve. Following analysis of such samples always multiply results by the appropriate dilution factor to produce actual final concentration.

The influence of various drugs on end results has not been investigated. Bacterial or fungal contamination and laboratory cross-contamination may also cause irregular results.

Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Washing Buffer, fill with Washing Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.

As with most biological assays conditions may vary from assay to assay therefore **a fresh standard curve must be prepared and run for every assay.**

12. Performance Characteristics

12.1. Sensitivity

The limit of detection of murine IL-15/IL-15R complex defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 2.0 pg/ml (mean of 6 independent assays).

12.2. Specificity

Recombinant murine IL-15 has a cross reactivity of 2% in this assay when spiked in excess (100 ng/ml).

The specificity of this ELISA has been confirmed with bone-marrow derived dendritic cells from IL-15 knock-out mice. Tissue culture supernatant from these cells following lipopolysaccharide (LPS) stimulation was negative for the presence of IL-15/IL-15R complex while wild type animals reveal more than 40 pg/ml.

12.3. Precision

Intra Assay
Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of murine IL-15/IL15R. 2 standard curves were run on each plate. Data below show the mean murine IL-15/IL15R concentration and the coefficient of variation for each sample (see Table 2).
The calculated overall intra-assay coefficient of variation was 5.3%.

Table 2
The mean of Murine IL-15/IL-15R Complex concentration and the coefficient of variation for each sample:

Sample	Experiment	Mean Murine IL-15/IL-15R Complex Concentration (pg/ml)	Coefficient of Variation (%)
1	1	269.47	2.4
	2	280.74	2.0
	3	295.31	2.8
2	1	192.93	6.5
	2	200.41	2.9
	3	217.88	1.8
3	1	163.07	5.6
	2	176.46	3.4
	3	188.24	5.1
4	1	100.26	3.8
	2	107.75	3.2
	3	94.11	3.5
5	1	43.17	3.4
	2	47.89	6.4
	3	46.66	4.9
6	1	19.83	8.5
	2	24.27	5.0
	3	22.24	5.4
7	1	18.42	5.2
	2	19.61	8.0
	3	19.57	10.4
8	1	14.34	8.2
	2	15.69	11.0
	3	16.37	8.5

Inter-assay
Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of Murine IL-15/IL-15R Complex. 2 standard curves were run on each plate. Data below show the mean Murine IL-15/IL-15R Complex concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 3).
The calculated overall inter-assay coefficient of variation was 6.3%.

Table 3
The mean of Murine IL-15/IL-15R Complex concentration and the coefficient of variation of each sample

Sample	Mean Murine IL-15/IL-15R Complex Concentration (pg/ml)	Coefficient of Variation (%)
1	281.84	4.6
2	203.74	6.3
3	175.93	7.2
4	100.70	6.8
5	45.91	5.3
6	22.11	10.0
7	19.20	3.5
8	15.47	6.7

12.4. Dilution Parallelism

Serum, plasma (EDTA) and cell culture supernatant samples with different levels of Murine IL-15/IL-15R Complex were analysed at serial 2 fold dilutions with 2 replicates each.

For data see Table 4.

Table 4

Sample matrix	Dilution	Recovery of Exp. Val. Mean (%)
Serum	1:4	92
	1:8	101
	1:16	110
Plasma (EDTA)	1:4	104
	1:8	117
	1:16	114
Cell culture supernatant	1:4	106
	1:8	105
	1:16	111

12.5. Spike Recovery

The spike recovery was evaluated by spiking 3 levels of murine IL-15/IL15R into serum, plasma (EDTA) and cell culture supernatant. Recoveries were determined with 2 replicates each. The amount of endogenous Murine IL-15/IL-15R Complex in unspiked samples was subtracted from the spike values.

For recovery data see Table 5

Table 5

Sample matrix	Spike high Mean (%)	Spike medium Mean (%)	Spike low Mean (%)
Serum	80	78	80
Plasma (EDTA)	71	71	66
Cell culture supernatant	102	111	128

12.6. Stability

Storage Stability
Aliquots of serum (spiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the Murine IL-15/IL-15R Complex level determined after 24h. There was no significant loss of Murine IL-15/IL-15R Complex immunoreactivity detected under above conditions.

Freeze-Thaw Stability
Aliquots of serum samples (spiked) were stored at -20°C and thawed 3 times, and the Murine IL-15/IL-15R Complex levels determined. There was no significant loss of Murine IL-15/IL-15R Complex immunoreactivity detected by freezing and thawing.

12.7. Expected Values

Panels of 40 serum as well as plasma samples (EDTA) from randomly selected donors (males and females) were tested for Murine IL-15/IL-15R Complex. For detected Murine IL-15/IL-15R Complex levels see Table 6.

Table 6

Sample Matrix	Number of Samples Evaluated	Mean (pg/ml)	Range (pg/ml)	Standard Deviation (pg/ml)
Serum	8	8.3	6.1 – 7.4	1.8
Plasma (EDTA)	4	6.7	6.3 – 7.4	0.5

13. Assay Summary

Total procedure length: 3h30mn

Wash two times

↓

Add sample, diluted standard and Biotin-Conjugate

↓

Incubate 2 hours at room temperature

↓

Wash four times

↓

Add 100µl of Streptavidin-HRP

↓

Incubate 1 hour at room temperature

↓

Wash four times

↓

Add 100 µl of ready-to-use TMB
Protect from light. Let the color develop for 30 mn.

↓

Add 100 µl Stop Reagent

↓

Read Absorbance at 450 nm

Supplier:

Diaclone SAS
6 Rue Dr Jean-François-Xavier Girod
25000 Besançon
France
Tel +33 (0)3 81 41 38 38
Email: techsupport@medixbiochemica.com