Neo Biotech

Human Adiponectin ELISA, High Sensitivity

NB-06-0156

Neo Biotech
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CONTENTS

Human Adiponectin ELISA, High Sensitivity

#Cat: NB-06-0156 Size: 1x96 Tests

1. INTENDED USE

The Human Adiponectin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human adiponectin. This kit is for research use only.

›› Features

For Research Use Only

- The total assay time is less than 3 hours.
- The kit measures total adiponectin in serum, plasma (EDTA, citrate, heparin).
- Assay format is 96 wells.
- Quality Controls are human serum based. No animal sera are used.
- Standards are human serum based.
- Components of the kit are provided ready to use or concentrated.

2. 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.

3. TEST PRINCIPLE

In the Neo Biotech Human Adiponectin ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human adiponectin antibody. After 60 minutes incubation and washing, polyclonal anti-human adiponectin antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with captured adiponectin. 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. The absorbance is proportional to the concentration of adiponectin. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

4. PRECAUTIONS

- **For Research Use Only.**
- Wear gloves and laboratory coats when handling biological materials.
- Do not drink, eat or smoke in the areas where biological 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.

- 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.

5. 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.

6. REAGENT SUPPLIED

Kit Components	*State*	*Quantity*
Antibody Coated Microtiter Strips	ready to use	96 wells
Conjugate Solution	ready to use	13 ml
Set of Standards	ready to use	8 x 1 mL
Quality Control High	concentrated	0.1 ml
Quality Control Low	concentrated	0.1 ml
Dilution Buffer	concentrated	20 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

7. 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 50-200 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)

8. 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.

- **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 desicant and seal carefully. Remaining Microtiter Strips are stable 3 months 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 months when stored at 2-8°C.

Quality Controls High, Low
Refer to the Certificate of Analysis for Quality Controls concentrations!!!
Quality Controls are ready to use, do not dilute them. (Quality Controls are supplied diluted 30x). Stability and storage
Opened Quality controls are stable 3 months when stored at 2-8°C.

- **Assay reagents supplied concentrated: Dilution Buffer Conc. (10x)**

Dilute only required amount of Dilution Buffer Concentrate. Otherwise dilute all 20 ml of Dilution Buffer Concentrate (10x) with 180 ml of distilled water to prepare 200 ml of Dilution Buffer (1x) for use of all-wells.

Stability and storage:
The diluted Dilution Buffer is stable 1 week when stored at 2-8°C. Opened Dilution Buffer Concentrate (10x) is stable 3 months when stored at 2-8°C.

Quality Controls HIGH, LOW

Refer to the Certificate of Analysis for current Quality Control concentration!!!

Dilute Quality Control (HIGH and LOW) 10x with the Dilution Buffer just prior to the assay, e.g. 30 ml of QC + 270 ml of Dilution Buffer for duplicates. (Quality Controls are supplied diluted 30x). **It means the final dilution is 300x and the concentration of Quality Control calculated from the standard curve must be multiplied by a dilution factor of 300.**

Mix well (not to foam). Vortex is recommended. Beware of imprecision in pipetting.

Stability and storage:

Opened Quality Controls are stable 3 months when stored at 2-8°C. Do not store the diluted Quality Controls

Note:

Concentration of analyte in Quality Controls need not be anyhow associated with normal and/or pathological concentrations in serum or another body fluid. Quality Controls serve just for control that the kit works in accordance with PDS and CoA and that ELISA test was carried out properly.

Wash Solution Conc. (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.

9. PREPARATION OF SAMPLES

The kit measures adiponectin in serum, plasma (EDTA), citrate, heparin, urine, cerebrospinal fluid (CSF) and breast milk.

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.

Serum or plasma samples:

Dilute serum or plasma 300x with the Dilution Buffer prior to the assay in two steps:

Dilution A (10x):

Add 10 l of samples to 90 l of Dilution Buffer. **Mix well** (not to foam).

Dilution B (30x):

Add 10 l of Dilution A into 290 l of Dilution Buffer to prepare final dilution (300x). **Mix well** (not foam).

One step-dilution can be performed (add 5 l of samples to 1495 l of Dilution Buffer). Beware of imprecision in pipetting and mix the samples very thoroughly!

Breast milk, urine and cerebrospinal fluid (CSF) samples:

Dilute samples 3x with Dilution Buffer just prior to the assay, e.g. add 100 l of sample to 200 l of Dilution Buffer for duplicates. **Mix well** (not to foam)

Stability and storage:

Samples should be stored at -20°, or preferably at -70°C for long-term storage. Avoid repeated freeze/ thaw cycles.
Stability of milk, urine and CSF samples have not been tested.
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 adiponectin.

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

10. ASSAY PROCEDURE

Adiponectin levels are significantly lower (2-3 orders of magnitude) in breast milk, urine or CSF than in serum or plasma. Therefore, different protocols have to be used.

Protocol (a) for serum and plasma samples: Sample dilution is 300x
Standard range is 5-100 ng/ml (the Standards of 150 ng/ml and/or 2 ng/ml can be added optionally)
Incubation with Substrate Solution is 10 minutes

Protocol (b) for breast milk, urine or CSF: Sample dilution 3x Standard range 1-50 ng/ml
Incubation with Substrate Solution is 25-30 minutes

The other assay procedure is same for both ELISA protocols.

1. Pipet **100 l** of Standards (5-100 ng/ml for serum or plasma samples, 1-50 ng/ml for milk, urine or CSF samples), diluted Quality Controls, Dilution Buffer (=Blank) and diluted 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 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add **100 l** 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 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add **100 l** 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** (serum or plasma samples) or **25-30 minutes** (milk, urine, and CSF samples) at room temperature (20-30°C). The incubation time may be extended [up to 20 minutes for serum and plasma samples or up to 50 minutes for milk urine and CSF samples] 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 l** 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).

Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm. **The absorbance should be read within 5 minutes following step 9.**
Note: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine adiponectin concentration of off- scale standards and samples. The readings at 405 nm should not replace the readings for samples that were "in range" 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 10**	**QC High**	Sample 7	Sample 15	Sample 23	Sample 31
B	**Standard 5**	**QC Low**	Sample 8	Sample 16	Sample 24	Sample 32
C	**Standard 2**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
D	**Standard 1**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
E	**Standard 0.5**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
F	**Standard 0.2**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
G	**Standard 0.1**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
H	Blank	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38

Figure 1: Example of a work sheet.

11. 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 adiponectin µg/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.

The measured concentration of Quality Controls calculated from the standard curve must be multiplied by a dilution factor of 300 and the measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because Quality Controls and samples have been diluted prior to the assay, e.g.
13.5 ng/ml (from standard curve) x 300 (dilution factor for serum and plasma samples) =
4.05 mg/ml

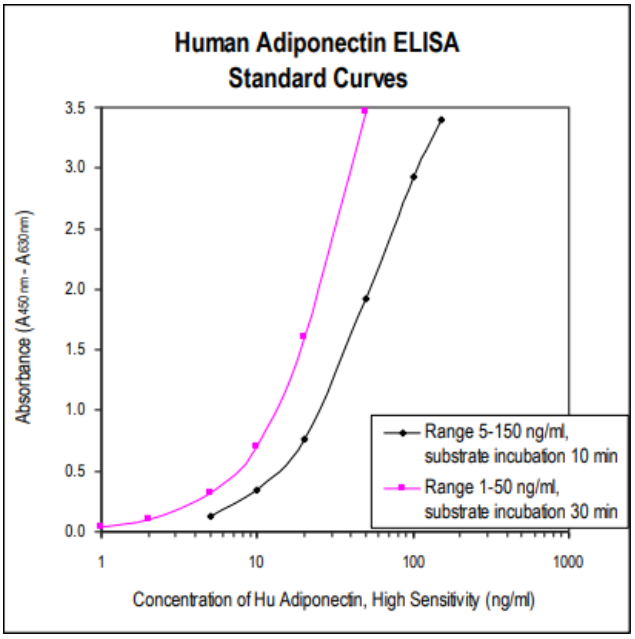

Figure 2: Typical Standard Curve for Human Adiponectin ELISA.

12. PERFORMANCE CHARACTERISTICS

Typical analytical data of Neo Biotech Human Adiponectin ELISA, High Sensitivity are presented in this chapter

Sensitivity
Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: Ablank + 3xSDblank) is calculated from the real adiponectin values in wells and is different for two protocols described in the paragraph 11:
For Protocol (a) (for serum or plasma samples) recommended sample dilution 300x, calibration range 5–150 ng/ml, substrate incubation 10 min: LOD is 0.47 ng/ml.
For Protocol (b) (for milk, CSF or urine samples) recommended sample dilution 3x, calibration range 1-50 ng/ml, substrate incubation 25-30 min: LOD is 0.156 ng/ml
*Dilution Buffer is pipetted into blank wells.

Limit of assay
Results exceeding the calibration range should be repeated with more diluted samples.
The samples with extremely high adiponectin levels can be diluted up to 2400x. Dilution factor needs to be taken into consideration in calculating the adiponectin concentration.

Specificity

The antibodies used in this ELISA are specific for human adiponectin. The assay recognizes natural and recombinant (full length, mutation-modified trimer-only-forming, and globular domain) human adiponectin.

No cross-reactivity has been observed for human leptin, leptin receptor and resistin at 100 ng/ml. Determination of adiponectin does not interfere with hemoglobin (0.25 mg/ml), bilirubin (85 umol/l) and triglycerides (2.5 mmol/l). Interference over 10% was measured at the higher concentrations.

Sera of several mammalian species were measured in the assay. See results below. For details please contact us at info@neo-biotech.com.

Mammalian serum sample	*Observed crossreactivity*
Bovine	no
Dog	no
Cat	no
Goat	no
Hamster	no
Horse	no
Monkey	yes
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	no

Precision

Intra-assay (Within-Run) (n=8)

Sample	*Mean (µg/ml)*	*SD (µg/ml)*	*CV (%)*
1	6.34	0.28	4.4
2	9.41	0.31	3.3

Inter-assay (Run-to-Run) (n=8)

Sample	*Mean (µg/ml)*	*SD (µg/ml)*	*CV (%)*
1	9.41	0.54	5.8
2	17.74	1.11	6.2

- **Spiking Recovery**

Serum samples were spiked with different amounts of human adiponectin and assayed.

Sample	***O**bserved (µg/ml)*	***E**xpected (µg/ml)*	*Recovery **O/E** (%)*
1	4.36 8.48 12.25 23.12	- 9.36 14.36 24.36	- 91 85 95
2	6.90 12.29 15.23 25.83	- 11.90 16.90 26.90	- 103 90 96

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (µg/ml)*	***E**xpected (µg/ml)*	*Recovery **O/E** (%)*
1	- 2x 4x 8x	14.79 7.87 3.56 1.84	- 7.40 3.70 1.85	- 106 96 100
2	- 2x 4x 8x	23.39 11.50 5.69 2.95	- 11.70 5.85 2.92	- 98 97 101

- **Effect of sample matrix**

EDTA, citrate and heparin plasmas were compared to respective serum samples from the same 10 individuals.

Results are shown below:

Volunteer No.	Serum *(µg/ml)*	Plasma *(µg/ml)*		
		EDTA	Citrate	Heparin
1	7.37	6.01	5.52	6.23
2	5.52	6.71	4.97	6.19
3	4.57	3.84	3.63	3.67
4	6.57	7.87	6.98	9.05
5	12.89	11.54	11.88	11.83
6	13.72	15.42	13.20	16.32
7	5.82	4.88	3.95	4.81
8	15.29	14.74	15.66	16.97
9	11.43	10.03	9.95	10.44
10	5.93	5.71	6.05	5.39
Mean (µg/ml)	**8.9**	**8.7**	**8.2**	**9.4**
Mean Plasma/Serum (%)	-	**97.4**	**91.8**	**105.6**
Correlation. coeff. R2	-	**0.92**	**0.96**	**0.91**

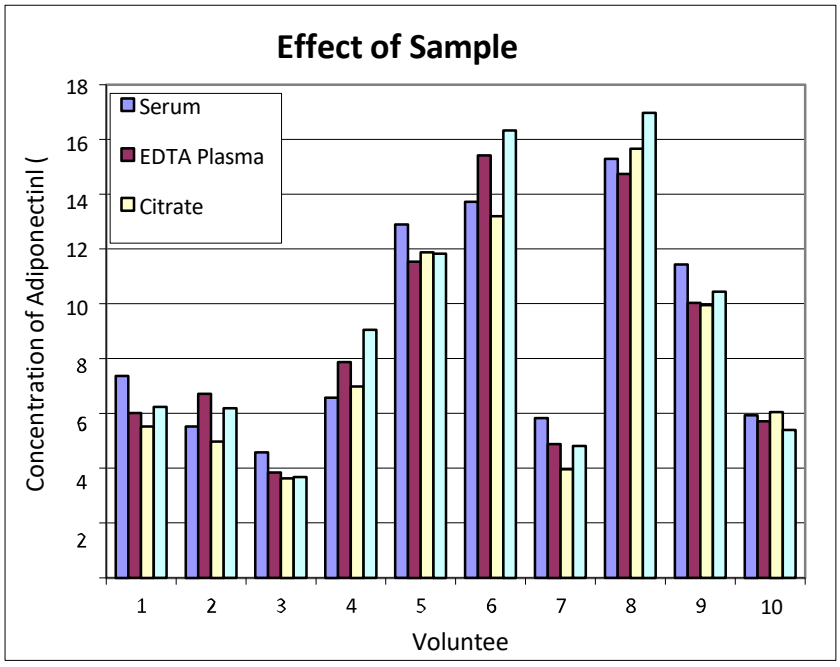

Fig. 3: Adiponectin levels measured using Human Adiponectin ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

- **Stability of samples stored at 2-8°C**

Samples should be stored at -20°C. However, no significant decline in concentration of human adiponectin was observed in serum and plasma samples after 7 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 *(µg/ml)*	Plasma *(µg/ml)*		
			EDTA	Citrate	Heparin
1	-20°C	2.01	2.08	1.79	1.16
	2-8°C, 1 day	2.07	1.89	1.69	1.85
	2-8°C, 7 days	1.86	1.89	1.64	1.67
2	-20°C	7.30	6.76	6.56	5.78
	2-8°C, 1 day	7.24	6.83	6.39	6.20
	2-8°C, 7 days	7.10	7.07	5.87	6.20
3	-20°C	10.72	15.13	11.75	11.02
	2-8°C, 1 day	10.99	13.65	12.36	10.89
	2-8°C, 7 days	12.16	13.38	10.59	10.48

- **Effect of Freezing/Thawing**

No significant decline was observed in concentration of human adiponectin 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 *(µg/ml)*	Plasma *(µg/ml)*		
			EDTA	Citrate	Heparin
1	1x	7.17	7.88	6.25	7.47
	3x	7.38	8.99	7.88	8.98
	5x	6.87	10.31	7.98	10.57
2	1x	10.86	13.16	10.83	10.60
	3x	13.53	14.47	13.21	11.51
	5x	11.23	11.22	8.64	10.96
3	1x	10.66	8.80	8.66	9.17
	3x	9.52	10.34	9.09	8.75
	5x	10.13	8.54	9.26	8.89

13. DEFINITION OF THE STANDARD

The recombinant human adiponectin is used as the Standard. The recombinant human adiponectin is produced in HEK293 cell line and contains 225 amino acid residues of the human adiponectin and 8 extra AA

14. 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:

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

» High coefficient of variation (CV)

Possible explanation:

d. Improper or inadequate washing
e. Improper mixing Standards, Quality Controls or samples

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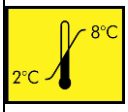
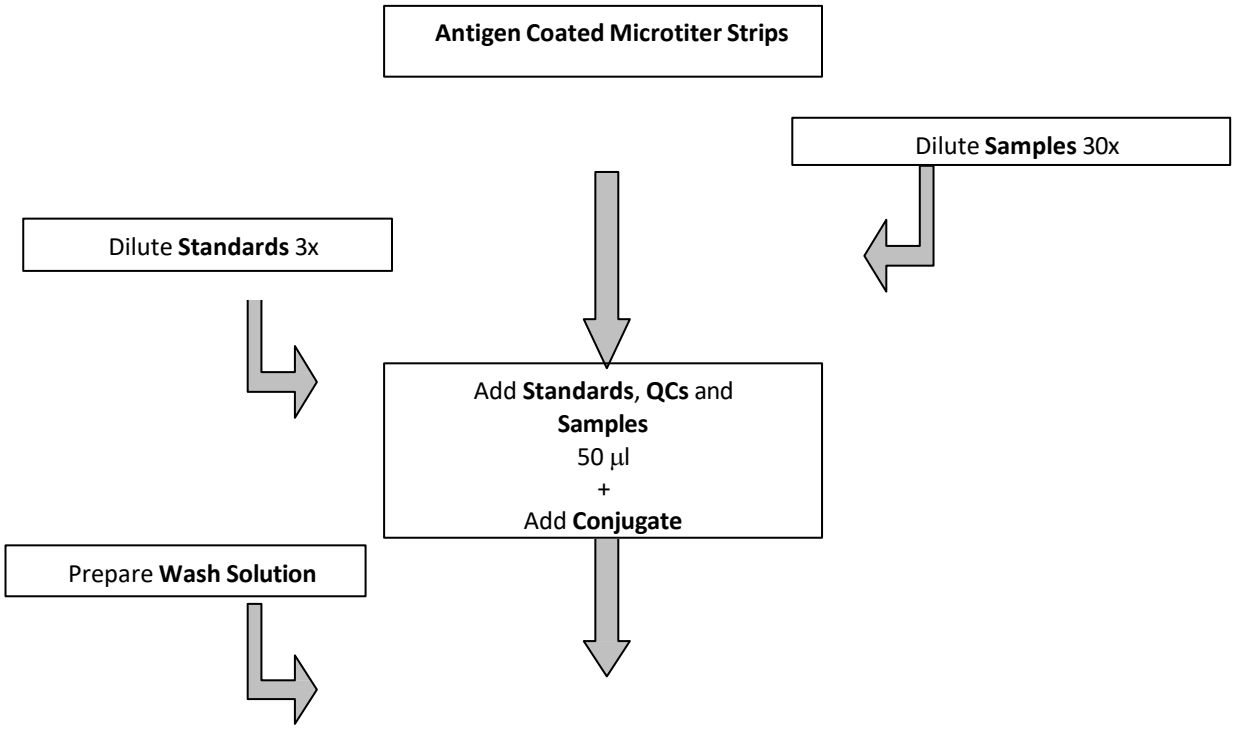
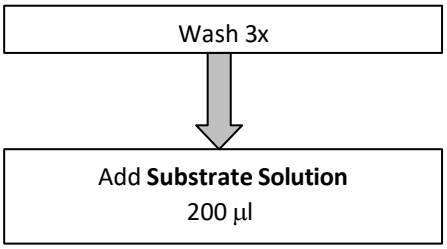
16. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	See instructions for use
	Biological hazard
	Expiry date
	Storage conditions
	Identification of packaging materials

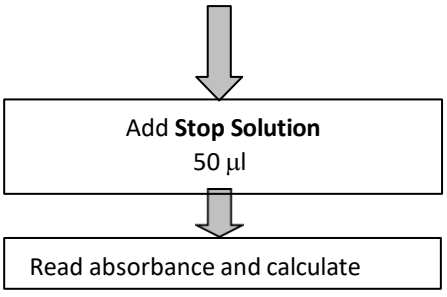
Assay Procedure Summary

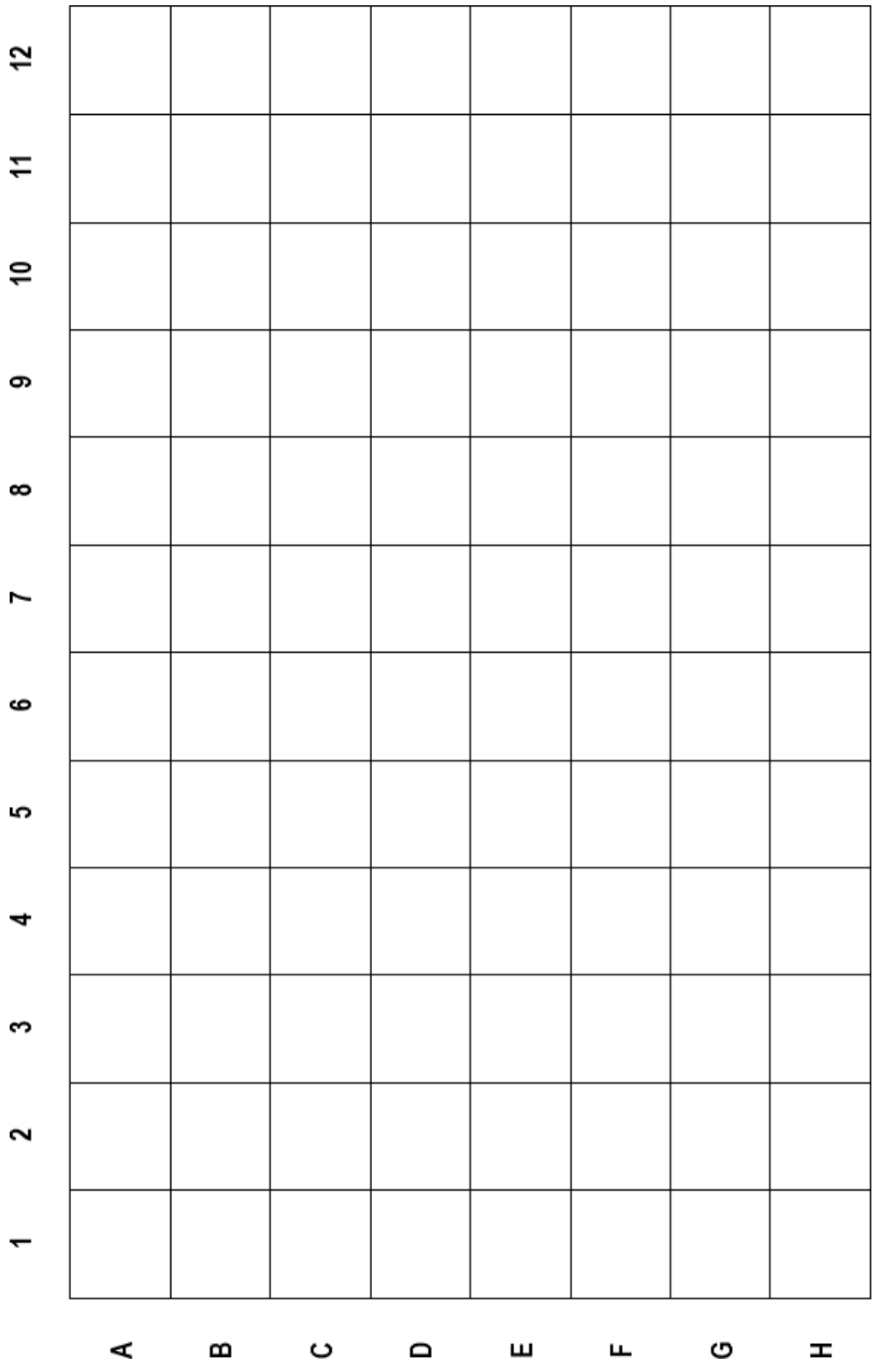


Incubate at RT for 2 hour/ 300rpm



Incubate at RT for 10-15 min.

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