

Mouse Adiponectin ELISA

Cat. No. RD293023100R

Manufacturer

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Use only the actual version of Product Data Sheet enclosed with the kit!

1. Intended Use

The RD293023100R Mouse Adiponectin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of mouse Adiponectin/Acrp30 protein in serum and tissue culture medium. It is intended for research use only.

Features

- The total assay time is less than 3 hours.
- The kit measures mouse serum Adiponectin/Acrp30.
- Quality Controls are mouse serum based.
- Components of the kit are provided ready-to-use, concentrated and lyophilized.

2. Storage, Expiration

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

3. Summary

Adiponectin, also referred to as Acrp30, AdipoQ and GBP-28, is a recently discovered 244 amino acid protein, the product of the *apM1* gene, which is physiologically active and specifically and highly expressed in adipose cells (adipokine). The protein belongs to the soluble defence collagen superfamily; it has a collagen-like domain structurally homologous with collagen VIII and X and complement factor C1q-like globular domain. Adiponectin forms homotrimers, which are the building blocks for higher order complexes found circulating in serum. Adiponectin receptors AdipoR1 and AdipoR2 have been recently cloned; AdipoR1 is abundantly expressed in skeletal muscle, whereas AdipoR2 is predominantly expressed in the liver.

Paradoxically, adipose tissue-expressed adiponectin levels are inversely related to the degree of adiposity. A reduction in adiponectin serum levels is accompanied by insulin resistance states, such as obesity and type 2 diabetes mellitus. It is also reported in patients with coronary artery disease. Increased adiponectin levels are associated with type 1 diabetes mellitus, anorexia nervosa and chronic renal failure. Adiponectin concentrations correlate negatively with glucose, insulin, triglyceride concentrations and body mass index and positively with high-density lipoprotein-cholesterol levels and insulin-stimulated glucose disposal.

Adiponectin has been shown to increase insulin sensitivity and decrease plasma glucose by increasing tissue fat oxidation. It inhibits the inflammatory processes of atherosclerosis suppressing the expression of adhesion and cytokine molecules in vascular endothelial cells and macrophages, respectively. This adipokine plays a role as a scaffold of newly formed collagen in myocardial remodelling after ischaemic injury and also stimulates angiogenesis by promoting cross-talk between AMP-activated protein kinase and Akt signalling in endothelial cells.

4. Test Principle

Surface of wells in microtitration plate is coated with monoclonal anti-mouse Adiponectin specific antibody. Standards, Quality Controls (QC) and diluted samples are pipetted into the wells. Any mouse Adiponectin/Acrp30 present is captured by immobilized antibody and unbound protein is washed away after the first incubation period. Then, a horseradish peroxidase (HRP) conjugated polyclonal anti-mouse Adiponectin antibody is added to the wells and incubated. Following another washing step, to remove unbound antibody-HRP conjugate, a substrate solution (H_2O_2 and TMB) is added to the wells. The enzymatic reaction yields a blue product that turns yellow when acidic stop solution is added. The intensity of the colour, measured spectrophotometrically at 450 nm, is directly proportional to the amount of the mouse Adiponectin bound in the initial step. Concentration of the diluted test samples are then read off the standard curve that is constructed by plotting the absorbance values against each respective mouse Adiponectin standard level, using a four-parameter function. The dilution factor needs to

be taken into consideration when calculating the actual concentration of Adiponectin/Acrp30 analyte in the test samples.

5. Precautions

- For research use only.
- This kit contains components of animal origin.
- Wear gloves, eye and clothing protection when handling supplied immunodiagnostic material, particularly acidic Stop Solution, and Substrate Solution that contains hydrogen peroxide and tetramethylbenzidine (TMB). Avoid contact with these two latter reagents. The Stop and/or Substrate Solutions may cause skin/eye irritation. In such a case, wash the skin/eyes thoroughly with water and seek medical attention if necessary.
- All solutions supplied must not be pipetted by mouth.
- Do not drink, eat or smoke in the area where immunodiagnostic materials are being handled.
- Do not mix reagents from different kit lots.
- Reagents should not be used beyond the expiration date marked on the kit label.

6. Reagents Supplied

<i>Cat. No.</i>	<i>Kit Components</i>	<i>Quantity</i>
C251211	Microtiter Strips, coated with Anti-Mouse Adiponectin Monoclonal Antibody, sealed	96 wells
C252211	Conjugate Solution, ready to use	13 ml
C253141	Mouse Adiponectin Master Calibrator, 8 ng	2 vials
C254151	Quality Control High, lyophilized	2 vials
C254251	Quality Control Low, lyophilized	2 vials
C005211	Dilution Buffer Concentrate (10x)	22 ml
C006111	Wash Solution Concentrate (5x)	100 ml
C007111	Substrate Solution (TMB), ready to use	13 ml
C008111	Stop Solution (0.2 M H ₂ SO ₄) ready to use	13 ml
-	Instruction Manual + Certificate of Analysis	1 pc

7. Materials Required but Not Supplied

- Test tubes for diluting samples
- Precision pipettes to deliver 10-1000 μ l and disposable tips
- Multichannel pipette 50-200 μ l
- Microplate reader with 450 ± 10 nm filter
- Orbital microplate shaker capable of agitation at approximately 300 rpm
- Software package facilitating data generation and analysis
- Microplate washer (optional) [Manual washing is possible but not preferable.]
- Glassware (graduated cylinder and bottle for Wash Solution)
- Distilled water

8. Preparation of Reagents

All reagents need to be brought to room temperature prior to the assay.

Assay reagents supplied ready to use:

Conjugate Solution, Substrate Solution (TMB), Stop Solution (0.2M H₂SO₄)

- If you do not use the whole plate, return unused strips in the provided aluminium bag with dessicant and seal the bag carefully. Keep the unused strips at 2-8°C, protected from the moisture.

Assay reagents supplied concentrated:

Wash Solution:

Dilute 100 ml of Wash Solution Concentrate (5x) with 400 ml of distilled water to prepare 500 ml of Wash Solution (1x).

Stability and storage:

The diluted Wash Solution (1x) is stable for 1 month when stored at 2-8°C.

Dilution Buffer:

Dilute only required amount of Dilution Buffer Concentrate.

Otherwise dilute all 22 ml of Dilution Buffer Concentrate (10x) with 198 ml of distilled water to prepare 220 ml of Dilution Buffer (1x).

Stability and storage:

The diluted Dilution Buffer (1x) is stable for 1 week when stored at 2-8°C.

Assay reagents supplied lyophilized:

Mouse Adiponectin Master Calibrator:

Reconstitute the lyophilized mouse Adiponectin (in 1 vial) with 1 ml of Dilution Buffer to prepare master calibrator stock solution. The produced concentration of the Adiponectin in the stock solution is 8 ng/ml. Mix gently the stock and allow it to sit for about 5 minutes optimally (to ensure complete reconstitution). Always avoid foaming when reconstituting or mixing the protein solutions.

Subsequent concentration levels prepare by serially dilution as follows:

<i>Master Calibrator Volume</i>	<i>Dilution Buffer</i>	<i>Concentration</i>
stock: 8 ng lyophilized	1 ml	8 ng/ml
0.5 ml of 8 ng/ml	0.5 ml	4 ng/ml
0.5 ml of 4 ng/ml	0.5 ml	2 ng/ml
0.5 ml of 2 ng/ml	0.5 ml	1 ng/ml
0.5 ml of 1 ng/ml	0.5 ml	0.5 ng/ml
0.5 ml of 0.5 ng/ml	0.5 ml	0.25 ng/ml

Mix each test tube before the next transfer.

The stock solution serves as the highest standard level (8 ng/ml) and the Dilution Buffer serves as the Blank in the assay.

Stability and storage:

Do not store diluted calibrator solutions.

Quality Controls:

Reconstitute the lyophilized High and Low Quality Control (1 vial each) with 1 ml of Dilution Buffer.

Assay these controls simply reconstituted (without further dilution).

Stability and storage:

Do not store the reconstituted Quality Controls.

9. Preparation of Samples

Dilute serum samples, just prior to perform the test, 1:10 000 with Dilution Buffer in two steps as follows:

Add 10 µl sample into 990 µl Dilution Buffer (dilution 1:100 and/or dilution A); mix well.

Pipet 10 µl of dilution 1:100 (dilution A) into another 990 µl Dilution Buffer to prepare final dilution 1:10 000 (dilution B). Mix well.

Stability and storage:

Do not store diluted samples.

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

- 1) Prepare reagents, calibrator dilutions, controls and samples as directed in the previous sections. Remove excess microplate strips from the plate frame.
- 2) Pipet 100 μ l of each individual concentration of Master Calibrator, Quality Controls, diluted samples (dilution B) and Dilution Buffer (=Blank), preferably in duplicates, into the appropriate wells.
See Figure 1 for example of work sheet.
- 3) Incubate the plate at room temperature (ca. 26°C) for 1 hour, shaking at about 300 rpm on the orbital microplate shaker.
- 4) Wash the wells 3-times with Wash Solution (0.35 ml per well). Invert the plate and blot it against paper towels to remove the remaining Wash Buffer.
- 5) Add 100 μ l of Conjugate Solution into each well.
- 6) Incubate the plate at room temperature (ca. 26°C) for 1 hour, shaking at about 300 rpm on the orbital microplate shaker.
- 7) Wash the wells 3-times with Wash Solution. Invert the plate and blot it.
- 8) Add 100 μ l of Substrate Solution. Protect from light! (Covering the plate with e.g. aluminium foil is recommended.)
- 9) Incubate the plate for **8-10 minutes** at room temperature. (The incubation time may be extended, if the reaction temperature is below 20°C.)
- 10) Stop the colour development by adding 100 μ l of Stop Solution.
- 11) Determine optical density in the plate by reading absorbances at 450 nm (within 15 minutes, following step 10).

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 mouse Adiponectin concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	Calibrator 8	Blank	Sample 8	Sample 16	Sample 24	Sample 32
B	Calibrator 4	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	Calibrator 2	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	Calibrator 1	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	Calibrator 0.5	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	Calibrator 0.25	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.

11. Calculations

Most microtiter plate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the absorbance (Y) of calibrators against the *log* of the known concentration (X) of calibrators, using the four-parameter function. Alternatively, the *logit log* function can be used to linearize the calibration curve (i.e. *logit* of absorbance (Y) is plotted against the *log* of the known concentration (X) of calibrators).

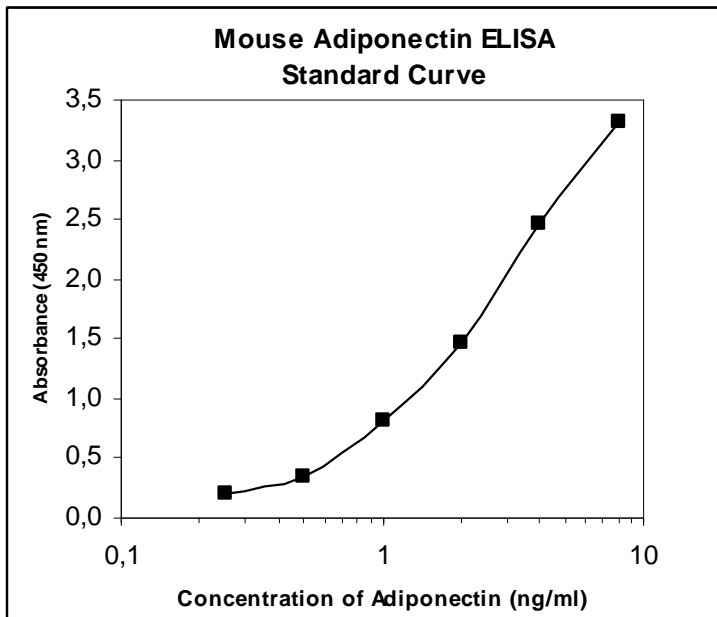


Figure 2: Standard Curve for mouse Adiponectin is plotted using the four-parameter function as a proportion of Adiponectin concentration and absorbance at 450 nm.

The concentration of Adiponectin in diluted samples is determined using a standard curve (e.g. the read value is 2.65 ng/ml). The actual amount of Adiponectin in the original serum has been assessed by multiplying the assay result by **dilution factor** 10 000 (e.g. 2.65 x 10 000 gives 26 500 ng/ml which represents 26.5 ug/ml).

12. Limits of Assay

Samples exceeding Adiponectin level of 8 ng/ml should be measured at higher dilution (e.g. 1:20 000) and a different dilution factor needs to be taken into consideration (20 000 in this case).

13. Performance Characteristics

Typical analytical data obtained with the BioVendor Mouse Adiponectin ELISA are presented below.

All the results in this chapter 13. are valid for 10 000-fold diluted samples and laboratory conditions of 26°C.

- **Sensitivity**

The limit of detection (defined as mouse Adiponectin concentration giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{\text{blank}} + 3 \times \text{SD}_{\text{blank}}$) is defined as follows:

Analytical Limit of Detection is calculated from the real Adiponectin values in wells and is 0.1ng/ml

Assay Sensitivity takes the dilution of samples into consideration and is calculated according to the formula:

Assay Sensitivity = Analytical Limit of Detection x sample dilution = 0.1ng/ml x 10 000 = 1ug/ml

*Dilution Buffer is pipetted into blank wells.

- **Specificity**

The antibodies in the Mouse Adiponectin ELISA kit are highly specific for mouse Adiponectin protein. No cross-reactivity with sera of other animal species (rat, hamster, rabbit, sheep, goat, cattle, swine, horse) has been observed.

The assay recognizes native and recombinant mouse Adiponectin. No cross-reactivity has been observed for mouse cytokines: RELM- α , RELM- β , Leptin, Leptin-receptor, Resistin; as well as for rat Leptin and human Adiponectin at 100 ng/ml.

Only fractional cross-reactivity 0.3% has been measured for rat recombinant adiponectin at 100 ng/ml.

- **Precision**

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

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	2.71	0.06	2.3
2	1.49	0.04	2.7
3	1.12	0.02	2.0
4	0.87	0.03	3.5

Inter-assay (Run-to-Run) (n=5)

<i>Sample</i>	<i>Mean (ng/ml)</i>	<i>SD (ng/ml)</i>	<i>CV (%)</i>
1	2.57	0.12	4.7
2	1.46	0.06	3.8
3	1.30	0.09	7.1
4	0.79	0.04	5.7

- **Spiking Recovery**

Serum samples were spiked to different levels of mouse Adiponectin, diluted with Dilution Buffer 1:10 000, and assayed.

<i>Sample</i>	<i>Addition</i>	<i>Observed (ng/ml)</i>	<i>Expected (ng/ml)</i>	<i>Recovery O/E (%)</i>
1	-	0.90	-	-
	0.5	1.34	1.40	98.5
	1.0	1.82	1.90	97.2
	2.0	2.65	2.90	97.8
2	-	2.00	-	-
	0.5	2.52	2.50	95.3
	1.0	3.09	3.00	95.0
	2.0	3.94	4.00	98.5

- **Dilution Linearity**

Serum samples were further serially diluted with Dilution Buffer, after primary dilution 1:10 000, and assayed.

<i>Sample</i>	<i>Dilution</i>	<i>Observed (ng/ml)</i>	<i>Expected (ng/ml)</i>	<i>Recovery O/E (%)</i>
1	-	2.61	-	-
	1 : 2	1.15	1.24	92.6
	1 : 4	0.51	0.62	82.4
	1 : 8	0.26	0.31	83.4
2	-	5.26	-	-
	1 : 2	2.55	2.63	96.8
	1 : 4	1.22	1.32	92.9
	1 : 8	0.53	0.66	80.7

- **Effect of freezing/thawing on the concentration of mouse Adiponectin in undiluted serum**

No significant decline was observed in concentration of mouse Adiponectin in serum samples after repeated (5x) freezing/thawing cycle. Analyte concentration varies within the limit of 10%.

Sodium azide and ϵ -aminocaproic acid are used as conservans.

However, we recommend to avoid using a sample more than once frozen and thawed.

Sample	Number of f/t Cycles	Serum	
		(ng/ml)	(%)
1	0	1.25	100.0%
	1x	1.24	99.4%
	3x	1.22	97.8%
	5x	1.24	99.5%
2	0	5.98	100.0%
	1x	6.21	103.9%
	3x	5.59	93.5%
	5x	5.63	94.3%
3	0	4.88	100.0%
	1x	5.42	111.0%
	3x	5.25	107.6%
	5x	4.95	101.5%
4	0	3.22	100.0%
	1x	3.41	106.1%
	3x	3.39	105.3%
	5x	3.51	109.1%

Mean values for 13 samples, in per cent units, are reported in the table below.

Number of Serum Samples	Number of f/t Cycles	Mean
n = 13	0	100.0%
n = 13	1x	97.5%
n = 13	3x	99.5%
n = 13	5x	100.6%

- **Stability of undiluted samples at 4°C**

No significant decline was observed in concentration of mouse Adiponectin in serum samples after 14 days storage at 4°C. Analyte concentration varies usually within the limit of 5%, rarely up to 15%.

There is no difference between alternative of serum with and without conservans.

Sodium azide and ε-aminocaproic acid are used as conservans.

In spite of this stability, we recommend to store samples frozen.

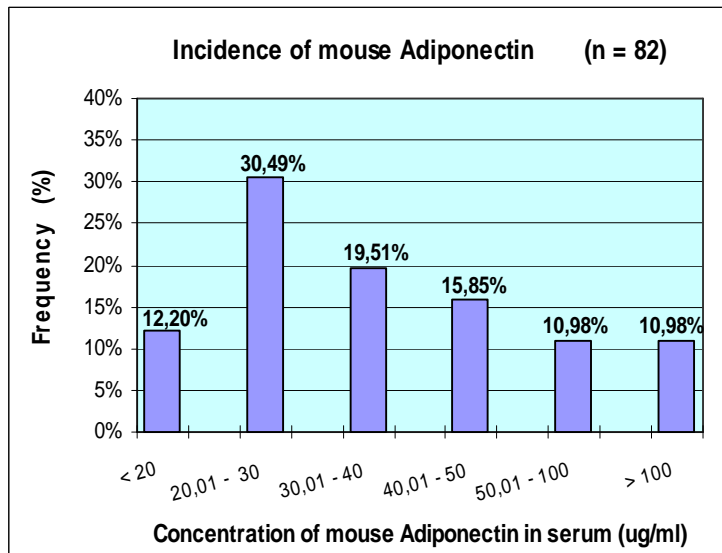
Sample	Temperature, Period	Serum	
		(ng/ml)	(%)
1	- 20°C	2.21	100.0%
	4°C, 1 day	2.11	95.2%
	4°C, 7 days	2.10	94.8%
	4°C, 14 days	2.14	96.7%
2	- 20°C	1.52	100.0%
	4°C, 1 day	1.42	93.0%
	4°C, 7 days	1.57	103.3%
	4°C, 14 days	1.49	97.4%
3	- 20°C	1.63	100.0%
	4°C, 1 day	1.51	92.5%
	4°C, 7 days	1.63	99.7%
	4°C, 14 days	1.72	105.6%
4	- 20°C	1.86	100.0%
	4°C, 1 day	1.89	101.8%
	4°C, 7 days	1.84	99.0%
	4°C, 14 days	1.89	101.6%

Mean values for 18 samples, in per cent units, are reported in the table below.

Number of Serum Samples	Temperature, Period	Mean
n = 18	- 20°C	100.0%
n = 18	4°C, 1 day	96.8%
n = 18	4°C, 7 days	102.3%
n = 18	4°C, 14 days	107.4%

14. Normal Values

The following values were obtained when 82 sera from healthy BALB/c mice were assayed:



15. Troubleshooting and FAQs

1/ 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

2/ High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time should be decreased before addition of Stop Solution

3/ High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing

16. References

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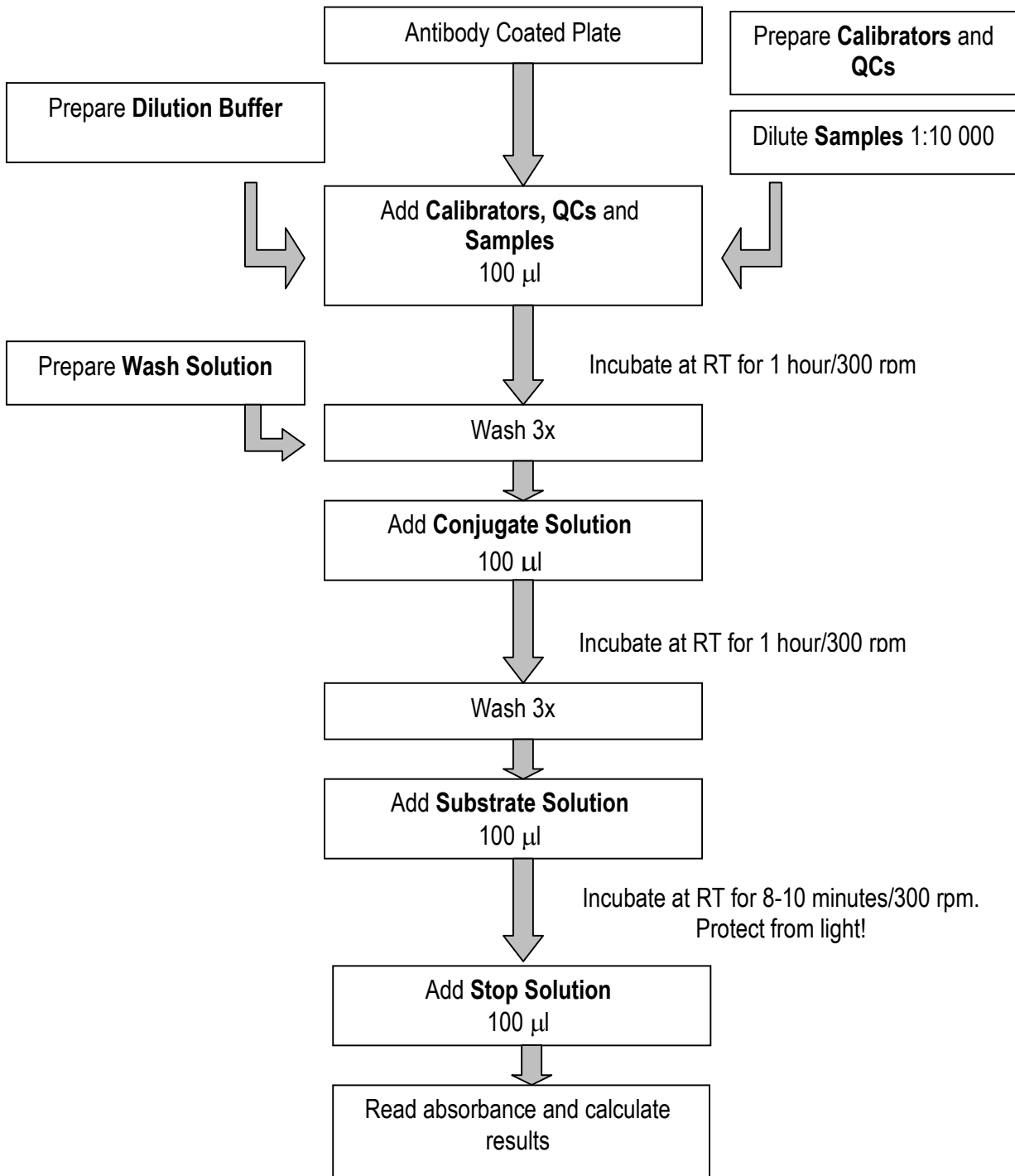
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