

# Rat Clusterin ELISA

Cat. No.: RD391034200R

***Manufacturer***

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

## **1. Intended Use**

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The RD391034200R Rat Clusterin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of rat Clusterin.

### **Features**

- It is intended for research use only.
- The total assay time is less than 3.5 hours.
- The kit measures Clusterin in rat serum and rat urine.
- Assay format – 96 wells
- Quality Controls are rat serum based. No human sera are used.
- Standard is recombinant protein based.
- Components of the kit are provided ready to use, concentrated or lyophilized.

## **2. Storage, Expiration**

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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. Introduction

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Clusterin (Apolipoprotein J; SP-40,40; TRPM-2; SGP-2; pADHC-9; CLJ; T64; GP III; XIP8) is a highly conserved disulfide-linked secreted heterodimeric glycoprotein of 75-80 kDa but truncated forms targeted to nucleus have also been identified.

Clusterin is highly conserved across species, showing 70-80% identity at the amino acid level amongst mammals, and numerous variants and isoforms have been describe. The protein is constitutively secreted by a number of cell types including epithelial and neuronal cells and is a major protein in physiological fluids including plasma, milk, urine, cerebrospinal fluid and semen.

Due to its wide breath of tissue distribution many diverse physiological functions have been attributed to clusterin including sperm maturation, membrane recycling, lipid transportation, tissue remodelling, complement inhibition and cell-cell or cell-substratum interactions. Moreover, it was propose, that clusterin functions is as an extra cellular chaperon that stabilizes stressed proteins in a folding-competent state and protein has also been implicated in programmed cell death. Another defining prominent of clusterin is its induction in many severe physiological disturbances states including kidney degenerative diseases, prostate and vesicle carcinogenesis, ovarian cancer and several neurodegenerative conditions.

Interesting study determine that urinary clusterin levels in the rat correlate with severity of tubular damage and may help to differentiate between glomerular and tubular injuries.

#### Areas of investigation:

Coronary heart diseases

Neurodegenerative diseases

Kidney degenerative disease

Renal tubular damage

#### **4. Test Principle**

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In the Biovendor Rat Clusterin ELISA, the Standards, Quality Controls and samples are incubated in microtiter plate wells pre-coated with polyclonal anti-rat Clusterin antibody. After 60 minutes incubation and a washing, biotin labelled polyclonal anti-rat Clusterin antibody is added and incubated with captured Clusterin for 60 minutes. After another washing, streptavidin-horseradish peroxidase (HRP) conjugate is added. After 30 minutes incubation and the last washing step, the remaining 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 Clusterin. A standard curve is constructed by plotting absorbance values against concentrations of Clusterin Standards, and concentrations of unknown samples are determined using this standard curve.

#### **5. Precautions**

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- Wear gloves and laboratory coats when handling immunodiagnostic materials.
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.
- This kit contains components of animal origin. 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.

## 6. Technical Hints

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- 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.

## 7. Reagents Supplied

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<i>Kit Components</i>	<i>State</i>	<i>Quantity</i>
Antibody Coated Microtiter Strips	sealed	96 wells
Biotin Labelled Antibody Concentrate (50x)	concentrated	0.26 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Rat Clusterin Master Standard	lyophilized	2 vials
Quality Control High	lyophilized	2 vials
Quality Control Low	lyophilized	2 vials
Biotin-Ab Diluent	ready to use	13 ml
Dilution Buffer	ready to use	50 ml
Wash Solution Concentrate (10x)	concentrated	100 ml
Substrate Solution (TMB)	ready to use	13 ml
Stop Solution (0.2 M H <sub>2</sub> SO <sub>4</sub> )	ready to use	13ml
Product Data Sheet + Certificate of Analysis	-	1 pc

## 8. Materials Required but Not Supplied

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- Deionized (distilled) water
- Test tubes for samples dilution
- Glassware (graduated cylinder and bottle) for Wash Solution
- Absorbent material (e.g. paper towels) for blotting the microtiter plate after washing

- Vortex mixer
- Precision pipettes to deliver 5-1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- 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)

## **9. Preparation of Reagents**

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**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-lock bag with desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months when stored at 2-8°C and protected from the moisture.

- **Dilution Buffer**
- **Biotin-Ab Diluent**
- **Straptavidin-HRP Conjugate**
- **Substrate Solution**
- **Stop Solution**

Stability and storage:

Opened reagents are stable 3 months when stored at 2-8°C.

Assay reagents supplied lyophilized or concentrated:

- **Rat Clusterin Master Standard:**

**IMPORTANT: Refer to the Certificate of Analysis for actual volume of Dilution Buffer needed for reconstitution of standard!!**

Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the Clusterin in the stock solution is **128 ng/ml**.

Prepare set of standards (128 ng/ml – 4 ng/ml) for measurement in serum samples or set of standards (128 ng/ml -2 ng/ml) for measurement in urine samples, using Dilution buffer as follows.

<i>Volume of Standard</i>	<i>Dilution Buffer</i>	<i>Concentration</i>
Stock	-	128 ng/ml
300 µl of stock	300 µl	64 ng/ml
300 µl of “ 64 ng/ml”	300 µl	32 ng/ml
300 µl of “ 32 ng/ml”	300 µl	16 ng/ml
300 µl of “ 16 ng/ml”	300 µl	8 ng/ml
300 µl of “ 8 ng/ml”	300 µl	4 ng/ml
300 µl of “ 4 ng/ml”	300 µl	2 ng/ml

Stability and storage:

The prepared Standards (128 - 4 or 2 ng/ml) must be used immediately. Do not store them.

- **Quality Controls High, Low**

**IMPORTANT: Refer to the Certificate of Analysis for actual volume of Dilution Buffer needed for reconstitution and for actual Quality Controls concentrations!!**

Reconstitute each Quality Control (High and Low) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).

Quality Controls are ready to use, do not dilute them.

Stability and storage:

Do not store reconstituted Quality Controls.

- **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.

- **Biotin Labelled Antibody Concentrate (50x)**

Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (50x) with 49 parts Biotin-Ab Diluent.

Example: 20 µl of “Biotin Labelled Antibody Concentrate (50x)” + 980 µl of Biotin-Ab Diluent for 1 strip (8 wells).

Stability and storage:

Do not store diluted Biotin Labelled Antibody solution.

Opened Biotin Labelled Antibody Concentrate (50x) is stable 3 months when stored at 2-8°C.

## **10. Preparation of Samples**

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The kit measures Rat Clusterin in serum and urine.

Samples should be assayed immediately after collection or should be stored frozen. 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 serum samples.

**Preparation of serum samples:**

Dilute samples just prior to the assay 2000x with Dilution Buffer in two steps as follows (for duplicates or singlets):

**Dilution A (40x):**

Add 5 µl sample into 195 µl Dilution Buffer and **mix well** (short gently vortex).

**Dilution B (50x):**

Add 5 µl of Dilution A into 245 µl Dilution Buffer to prepare final dilution 2000x and **mix well** (short gently vortex).

Stability and storage:

**Do not store the diluted samples.**

Serum samples should be stored at -20°C or preferably at -70°C for long term. Repeated freezing and thawing of the samples 5-times does not affect clusterin level.

**Preparation of urine samples:**

Dilute urine samples just prior to perform the test 10-fold with Dilution Buffer, e.g. 15 µl of sample + 135 µl of Dilution Buffer for singlets or 25 µl of sample + 225 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:

**Do not store the diluted samples.**

Urine samples should be stored at -70°C. At this condition samples are stable minimally 3 months. It is strongly recommended to avoid repeated freezing/thawing of the samples.

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

## 11. Assay Procedure

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- 1) Pipet 100  $\mu$ l of Standards, Quality Controls and diluted samples (Dilution B – for serum 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).
- 4) Add 100  $\mu$ l of Biotin Labelled Antibody 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).
- 7) Add 100  $\mu$ l of Streptavidin-HRP Conjugate.
- 8) Incubate the plate at room temperature (ca. 25°C) for 30 min, shaking at ca. 300 rpm on an orbital microplate shaker.
- 9) Wash the wells 3-times with Wash Solution (0.35 ml per well).
- 10) Add 100  $\mu$ l of Substrate Solution. (Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.)
- 11) Incubate the plate for 10 minutes for measurement in serum samples or 20 minutes for measurement in urine samples at room temperature (the incubation time may be extended [up to 20 or 30 minutes] if the reaction temperature is below than 20°C).  
No shaking!
- 12) Stop the colour development by adding 100  $\mu$ l of Stop Solution.
- 13) Determine the absorbance by reading the plate at 450 nm. (The absorbance should be read within 10 minutes following step 12.)

*Note 1: 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 Rat Clusterin 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 128	Blank	Sample 8	Sample 16	Sample 24	Sample 32
B	Standard 64	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	Standard 32	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	Standard 16	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	Standard 8	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	Standard 4	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 work sheet .

## 12. Calculations

Most microtiter plate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the absorbance (Y) of standards against the *log* of the known concentration (X) of standards, using the four-parameter function.

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

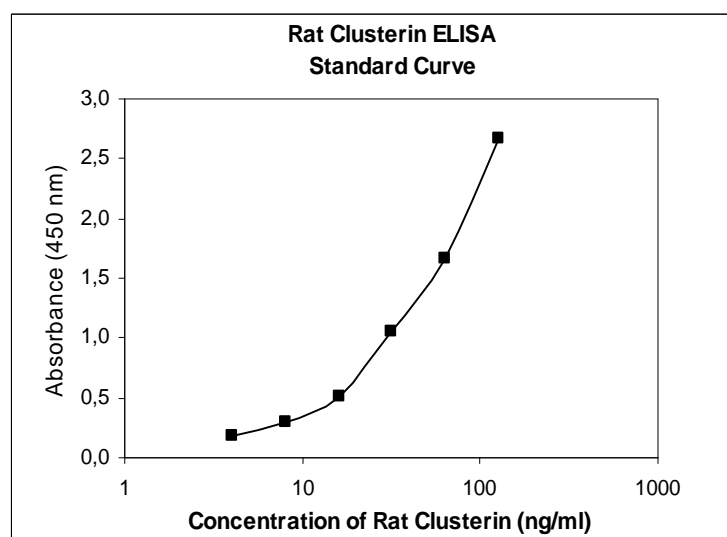


Figure 2: Typical Standard Curve for Rat Clusterin

Because samples have been diluted prior to the assay, the measured concentration must be multiplied by their respective dilution factors.

### 13. Performance Characteristics

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Typical analytical data obtained with the BioVendor Rat Clusterin ELISA are presented below. **For actual standard curve see the Certificate of Analysis.**

- **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:  $A_{\text{blank}} + 3xSD_{\text{blank}}$ ) is 0.7 ng/ml.

\* Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding Clusterin level of 128 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the Clusterin concentration.

- **Specificity**

The antibodies used in this ELISA kit are specific for rat Clusterin.

Human serum and sera of several mammalian species were measured in the assay. See results below.

For details please contact us at [info@biovendor.com](mailto:info@biovendor.com).

<i>Serum sample</i>	<i>Observed crossreactivity</i>
Bovine	no
Goat	no
Hamster	yes
Human	no
Horse	no
Monkey	no
Mouse	yes
Pig	no
Rabbit	no
Rat	no
Sheep	no

- **Precision**

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

<i>Sample</i>	<i>Mean (<math>\mu\text{g/ml}</math>)</i>	<i>Standard Deviation (<math>\mu\text{g/ml}</math>)</i>	<i>CV (%)</i>
1	31.6	1.23	3.9
2	37.6	1.81	4.8

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

<i>Sample</i>	<i>Mean (<math>\mu\text{g/ml}</math>)</i>	<i>Standard Deviation (<math>\mu\text{g/ml}</math>)</i>	<i>CV (%)</i>
1	29.3	1.61	5.5
2	37.6	2.33	6.2

- **Spiking Recovery**

Serum samples were spiked with different amounts of Rat Clusterin and assayed.

<i>Sample</i>	<i>Observed (<math>\mu\text{g/ml}</math>)</i>	<i>Expected (<math>\mu\text{g/ml}</math>)</i>	<i>Recovery O/E (%)</i>
1	26.9	-	-
	130.9	129.3	101.2
	76.9	78.1	98.5
	54.0	52.5	102.9
2	37.2	-	-
	90.6	101.2	89.5
	65.6	69.2	94.8
	52.0	53.2	97.7

- **Dilution Linearity**

Serum samples were diluted with Dilution Buffer and assayed.

<i>Sample</i>	<i>Dilution</i>	<i>Observed</i> ( $\mu\text{g/ml}$ )	<i>Expected</i> ( $\mu\text{g/ml}$ )	<i>Recovery</i> <b>O/E (%)</b>
1	-	30.9	-	-
	2x	15.2	15.5	98.4
	4x	8.3	7.7	107.4
	8x	3.7	3.9	95.8
2	-	41.7	-	-
	2x	20.1	20.9	96.4
	4x	10.5	10.4	100.7
	8x	5.3	5.2	101.7

#### **14. Definition of the Standard**

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The recombinant Rat Clusterin is used as the Standard. The recombinant Rat Clusterin, produced in *E.coli*, is 26.5 kDa protein containing 215 amino acid residues of the Rat Clusterin and 25 additional amino residues. The amino acid sequence of the recombinant Rat Cluster is 100% homologous to the amino acid sequence 146-360 of the Rat Clusterin precursor.

## **15. Troubleshooting and FAQs**

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### **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
- Improper wavelength when reading absorbance

### **2/ 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

### **3) High coefficient of variation (CV)**

Possible explanation:

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

## 16. References

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- Min BH, Kim BM, Lee SH, Kang SW, Bendayan M. and Park IS: Clusterin expression in the early process of pancreas regeneration in the pancreatectomized Rat. *The J of Histochem & Cytochem*, 2003, 51(10): 1355-1365
- Trougokos IP, Gonos ES: Functional analysis of clusterin/apolipoprotein J in cellular death induced by severe genotoxic stress. *Ann NZ Acad Sci*, 2004 Jun, 19:206-210

For more references on this product  
see our WebPages at [www.biovendor.com](http://www.biovendor.com)

## 17. EXPLANATION OF SYMBOLS

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**REF**

Catalogue number

**Cont.**

Content

**LOT**

Lot number



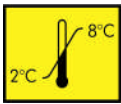
See instructions for use



Biological hazard



Expiry date

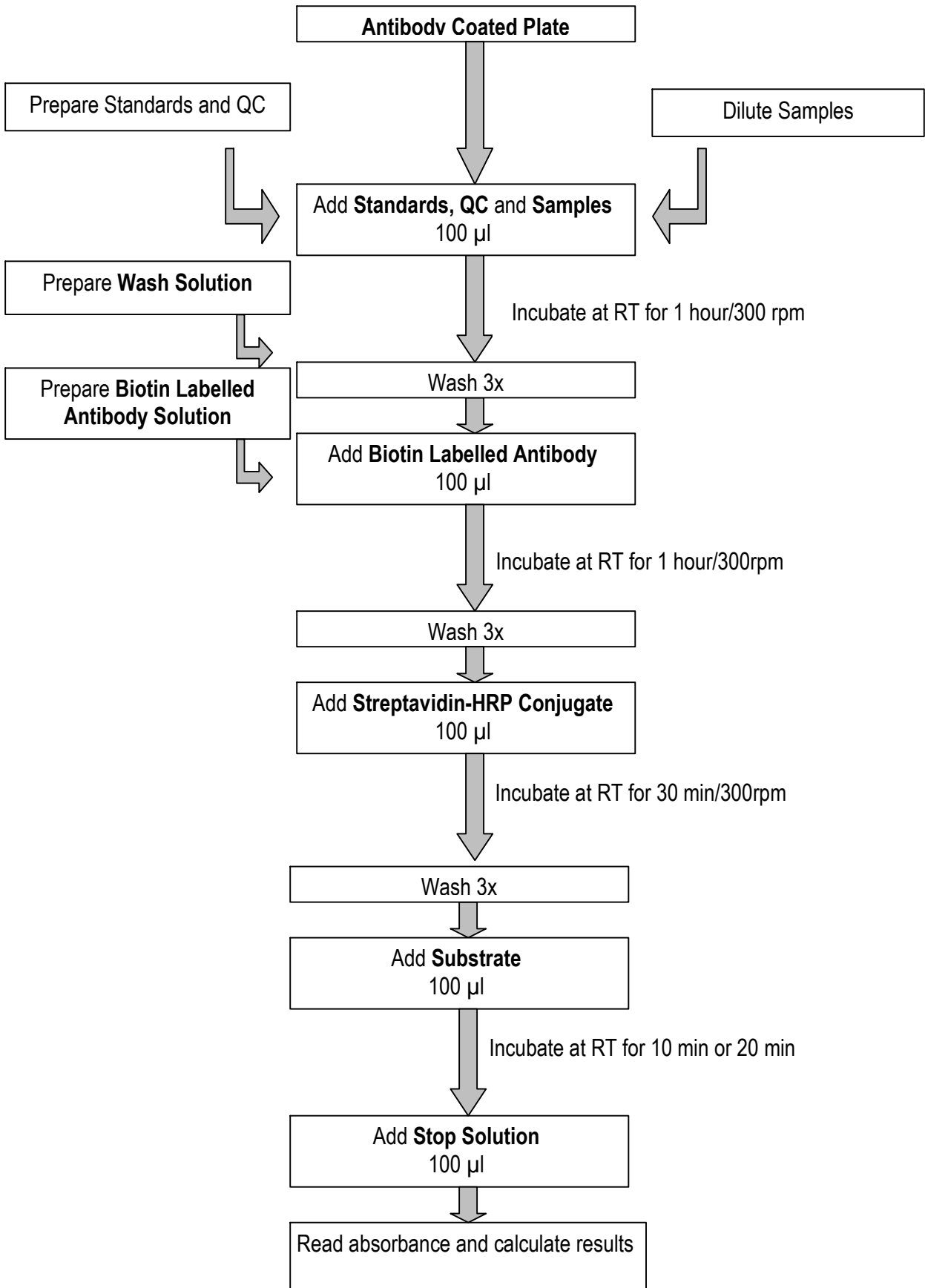


Storage conditions



Identification of packaging materials

### Assay Procedure Summary



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1


A B C D E F G H

**Notes:**

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