Version 1 Last updated 12 November 2020

Ab278042
Human CRP CatchPoint® SimpleStep ELISA® Kit (C-Reactive Protein)

For the quantitative measurement of CRP in human serum, plasma, and cell supernatant.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

CRP *in vitro* CatchPoint SimpleStep ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of CRP protein in human serum, plasma, and cell supernatant.

The CatchPoint SimpleStep ELISA employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. CatchPoint HRP Development Solution containing the Stoplight Red Substrate is added. During incubation, the substrate is catalyzed by HRP generating a fluorescent product. Signal is generated proportionally to the amount of bound analyte and the intensity is measured in a fluorescence plater reader at 530/570/590 nm Excitation/Cutoff/Emission.

C-reactive protein (CRP) displays several functions associated with host defense: it promotes agglutination, bacterial capsular swelling, phagocytosis and complement fixation through its calcium-dependent binding to phosphorylcholine. CRP can interact with DNA and histones and it may scavenge nuclear material released from damaged circulating cells. CRP is secreted; it forms a homopentamer pentaxin (or pentraxin) which have a discoid arrangement of 5 non-covalently bound subunits. CRP binds 2 calcium ions per subunit. The concentration of CRP in plasma increases greatly during acute phase response to tissue injury, infection or other inflammatory stimuli. It is induced by IL1/interleukin-1 and IL6//interleukin-6. This kit requires the use of acid treatment for the disassociation of CRP.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 50 µL standard or sample to appropriate wells

Add 50 µL Antibody Cocktail to all wells

Incubate at room temperature for 1 hour

Aspirate and wash each well three times with 350 µL 1X Wash Buffer PT

Add 100 µL of prepared CatchPoint HRP Development Solution to each well and incubate for 10 minutes

Read fluorescence at Ex/Cutoff/Em 530/570/590 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.
- All data, except Typical Standard Curve and Sensitivity were collected using the colorimetric version of this kit (ab278042).

6. Materials Supplied

Item	Quantity	Storage Condition
Human CRP Capture Antibody 10X	600 µL	+4°C
Human CRP Detector Antibody 10X	600 µL	+4°C
Human CRP Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 4BI	6 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
Stoplight Red Substrate Buffer	12 mL	+4°C
100X Stoplight Red Substrate	120 µL	+4°C
500X Hydrogen Peroxide (H_2O_2, 3%)	50 µL	+4°C
Sample Diluent NS	50 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Fluorescence microplate reader Ex/Cutoff/Em 530/570/590 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Hydrochloric acid (HCl)
- Sodium Hydroxide (NaOH)
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.

- When generating positive control samples, it is advisable to change pipette tips after each step.
- The provided Antibody Diluents and Sample Diluents contain protease inhibitor aprotinin. Additional protease inhibitors can be added if required.
- The incubation times provided in this protocol were optimized for fastest results with good signal. It is possible to increase the signal with longer incubation times, further optimization might be necessary.
- Keep in mind any RFU values shown are relative, NOT absolute. RFU from one plate reader are not comparable to another, especially if different make or model.
- To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 1X Wash Buffer PT:

Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

9.2 Antibody Cocktail:

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 4BI. Mix thoroughly and gently.

9.3 CatchPoint HRP Development Solution

Just prior to use prepare CatchPoint HRP Development Solution by diluting the 100X Stoplight Red Substrate and the 500X Hydrogen Peroxide in Stoplight Red Substrate Buffer.

For example, to make 6 mL of the CatchPoint HRP Development Solution combine 60 µL 100X Stoplight Red Substrate and 12 µL of 500X Hydrogen Peroxide with 5.928 mL Stoplight Red Substrate Buffer. Mix thoroughly and gently.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Reconstitute the CRP standard sample by adding 500 µL of Sample Diluent NS. Mix thoroughly and gently. Hold at room temperature for 10 minutes and mix gently. This is the 800 ng/mL **Stock Standard** Solution.

Acid Treatment Protocol: For dissociation of CRP in standard.

1) Dilute CRP Stock Standard to 40 ng/mL: Add 20 µL of CRP Stock Standard to 380 µL of Sample Diluent NS.
2) Add 50 µL of 0.25N HCL to serum. Incubate for 15 minutes at room temperature while rotating.
3) Add 50 µL of 0.25N NaOH to acid treated sample. Incubate for 3 minutes at room temperature while rotating.
4) CRP Stock Standard after treatment is 20 ng/mL, dilute treated CRP Stock Standard in Sample Diluent NS to prepare dilution series.

10.2 Label 14 tubes, Standards 1– 14.

10.3 Add 376 µL Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-14.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #14 contains no protein and is the Blank control.

Standards will be added to the plate in step 13.3. If desired all 14 standards can be used for a full standard curve. Alternatively, to commit fewer wells to the standard curve, select a subset of at least 7 standards plus the blank control. If 7 standards are desired, we recommend standards #3-9.

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Stock	192	208	20,000	9,600
2	Standard#1	150	150	9,600	4,800
3	Standard#2	150	150	4,800	2,400
4	Standard#3	150	150	2,400	1,200
5	Standard#4	150	150	1,200	600
6	Standard#5	150	150	600	300
7	Standard#6	150	150	300	150
8	Standard#7	150	150	150	75
9	Standard#8	150	150	75	37.5
10	Standard#9	150	150	37.5	18.75
11	Standard#10	150	150	18.75	9.38
12	Standard#11	150	150	9.38	4.69
13	Standard#12	150	150	4.69	2.34
14	none	0	150	0	0

11. Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	1:16,000 - 1:1000
Plasma - Citrate	1:32,000 - 1:2000
Plasma - EDTA	1:16,000 - 1:1000
Plasma - Heparin	1:16,000 - 1:1000
PBMC Cell culture supernatant*	< 25%

*Based on spiked sample

11.1 Plasma:

Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes Store un-diluted/untreated plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Acid Treatment Protocol: For dissociation of CRP in plasma samples.

1) Dilute plasma to 10%: Add 10 µL of plasma to 90 µL of Sample Diluent NS.
2) Add 50 µL of 0.25N HCL to 10% plasma. Incubate for 15 minutes at room temperature while rotating.
3) Add 50 µL of 0.25N NaOH to acid treated sample. Incubate for 3 minutes at room temperature while rotating.
4) Plasma concentration after treatment is 5%, dilute treated plasma sample in Sample Diluent NS to in assay concentration (see typical dynamic range).

11.2 Serum:

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Store un-diluted/untreated serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Acid Treatment Protocol: For dissociation of CRP in serum samples.

5) Dilute serum to 10%: Add 10 µL of serum to 90 µL of Sample Diluent NS.
6) Add 50 µL of 0.25N HCL to serum. Incubate for 15 minutes at room temperature while rotating.
7) Add 50 µL of 0.25N NaOH to acid treated sample. Incubate for 3 minutes at room temperature while rotating.
8) Serum concentration after treatment is 5%, dilute treated serum sample in Sample Diluent NS to in assay concentration (see typical dynamic range).

11.3 Cell Culture Supernatants:

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store un-diluted/untreated samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Acid Treatment Protocol: For dissociation of CRP in supernatant samples.

1) Add 50 µL of 0.25N HCL to 100 µL of neat cell culture supernatant. Incubate for 15 minutes at room temperature while rotating.
2) Add 50 µL of 0.25N NaOH to acid treated sample. Incubate for 3 minutes at room temperature while rotating.
3) Supernatant concentration after treatment is 50%, dilute treated supernatant sample in Sample Diluent NS to in assay concentration (see typical dynamic range).

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well fluorescence or "edge effects" have not been observed with this assay.
- Ensure plate and all materials are equilibrated to room temperature during use.
- Cover the plate with a plate seal during incubation steps.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.7 Add 100 µL of prepared CatchPoint HRP Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm. Further optimization of incubation time vs signal strength can be performed if needed.

13.8 Record the fluorescence at Ex/Cutoff/Em 530/570/590 nm. If using a Molecular Devices' plate reader supported by SoftMax® Pro software, a preconfigured protocol for these CatchPoint SimpleStep ELISA Kits is available with all the protocol and analysis settings at www.softmaxpro.org.

Mode:	Fluorescence
Instrument settings:	Endpoint
Excitation:	530 nm
Cutoff:	570 nm
Emission:	590 nm
Sensitivity:	6 flashes/read or 200ms
PMT:	Auto
Auto calibrate:	On
Read:	Top
Read Height:	1*

*For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

13.9 Analyze the data as described below.

14. Calculations

14.1 Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices

14.2 Calculate the average fluorescence value for the blank control (zero) standards. Subtract the average blank control standard fluorescence value from all other fluorescence values.

14.3 Create a standard curve by plotting the average blank control subtracted fluorescence value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

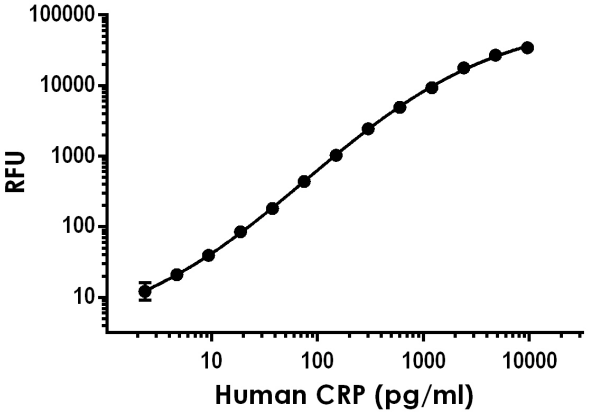
Δ Note: Most fluorescence reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.4 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted fluorescence values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

14.5 Samples generating fluorescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at fluorescence values less than that of the lowest standard should be retested in a less dilute form.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements			
Concentration (pg/mL)	RFU		Mean RFU
	1	2	
0	24	26	25
2.34	35	40	38
4.69	45	47	46
9.38	63	66	65
18.8	107	114	111
37.5	206	209	208
75.0	461	470	465
150	1091	1028	1060
300	2467	2492	2480
600	4857	5055	4956
1,200	9434	9320	9377
2,400	17851	17936	17893
4,800	26989	27162	27076
9,600	34210	34457	34333

Figure 1. Example of human CRP standard curve in Sample Diluent NS. The CRP standard curve was prepared as described in Section 10. Raw data generated on SpectraMax M4 Multi-Mode Microplate Reader is shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. Typical Sample Values

SENSITIVITY –

The calculated minimal detectable dose (MDD) is 5.36 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=22) and adding 2 standard deviations then extrapolating the corresponding concentration.

RECOVERY –

Three concentrations of CRP recombinant protein were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
1:4000 Serum	109	105 - 112
1:4000 Plasma - Citrate	107	105 - 109
1:4000 Plasma - EDTA	110	107 - 113
1:4000 Plasma - Heparin	114	112 - 119
25% PBMC Cell culture supernatant	100	99 - 100

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native CRP was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	1:1000 Human Serum	1:2000 Human Plasma (Citrate)	1:1000 Human Plasma (EDTA)	1:1000 Human Plasma (Heparin)
Undiluted	pg/mL	1049.0	865.9	707.4	1102.8
	% Expected value	100	100	100	100
2	pg/mL	526.4	426.1	331.9	551.1
	% Expected value	100	98	94	100
4	pg/mL	253.4	194.2	166.6	263.3
	% Expected value	97	90	94	96
8	pg/mL	125.4	102.3	83.2	126.8
	% Expected value	96	95	94	92
16	pg/mL	63.0	54.9	39.9	65.1
	% Expected value	96	101	90	94

Recombinant CRP was spiked into the following biological samples and diluted in a 2-fold dilution series in Sample Diluent NS.

Dilution Factor	Interpolated value	25% PBMC
Undiluted	pg/mL	587.8
	% Expected value	100
2	pg/mL	286.6
	% Expected value	98
4	pg/mL	139.9
	% Expected value	95
8	pg/mL	74.2
	% Expected value	101
16	pg/mL	39.9
	% Expected value	109

PRECISION –

Mean coefficient of variations of interpolated values of CRP from a single concentration of human serum within the working range of the assay.

	Intra-Assay	Inter-Assay
n =	8	3
CV(%)	1.4	4.0

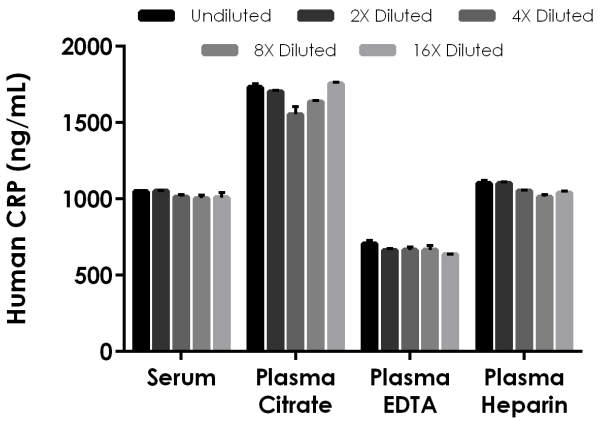


Figure 2. Interpolated concentrations of native CRP in human serum and plasma samples. The concentrations of CRP were measured in duplicates, interpolated from the CRP standard curves and corrected for sample dilution. Undiluted samples are as follows: serum 1:1000, plasma (citrate) 1:2000, plasma (EDTA) 1:1000, plasma (heparin) 1:1000. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean CRP concentration was determined to be 1025.5 ng/mL in serum, 1676.5 ng/mL in plasma (citrate) and 668.3 ng/mL in plasma (EDTA), and, 1062.8 ng/mL in plasma (heparin).

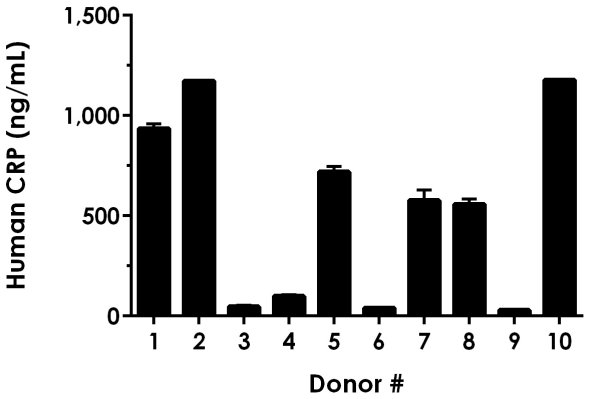


Figure 3. Serum from ten individual healthy human male donors was measured in duplicate. Interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean CRP concentration was determined to be 535.7 ng/mL with a range of 28.1 – 1180.4 ng/mL.

17. Assay Specificity

This kit recognizes both native and recombinant human and monkey CRP protein in serum, plasma, and cell culture supernatant.

Cell and tissue extract, urine, milk, and saliva samples have not been tested with this kit.

CROSS REACTIVITY

Recombinant mouse CRP and rat CRP were both prepared at 50 ng/mL respectively and assayed for cross reactivity. No cross-reactivity was observed.

INTERFERENCE

Recombinant mouse CRP and rat CRP were both prepared at 50 ng/mL respectively and tested for interference. No interference with was observed.

18. Species Reactivity

This kit recognizes human and monkey CRP protein.

Other species reactivity was determined by measuring 1:1000 serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

Reactivity < 3% was determined for the following species:

- Mouse
- Rat
- Cow

Other species reactivity not determined.

Please contact our Technical Support team for more information.

19. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with CatchPoint HRP Development Solution too brief	Read plate again after longer incubation time
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep Stoplight Red Substrate protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.

20. Notes

Technical Support

 This product was released in collaboration with Molecular Devices LLC, however for technical support questions, please contact Abcam Representatives.

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