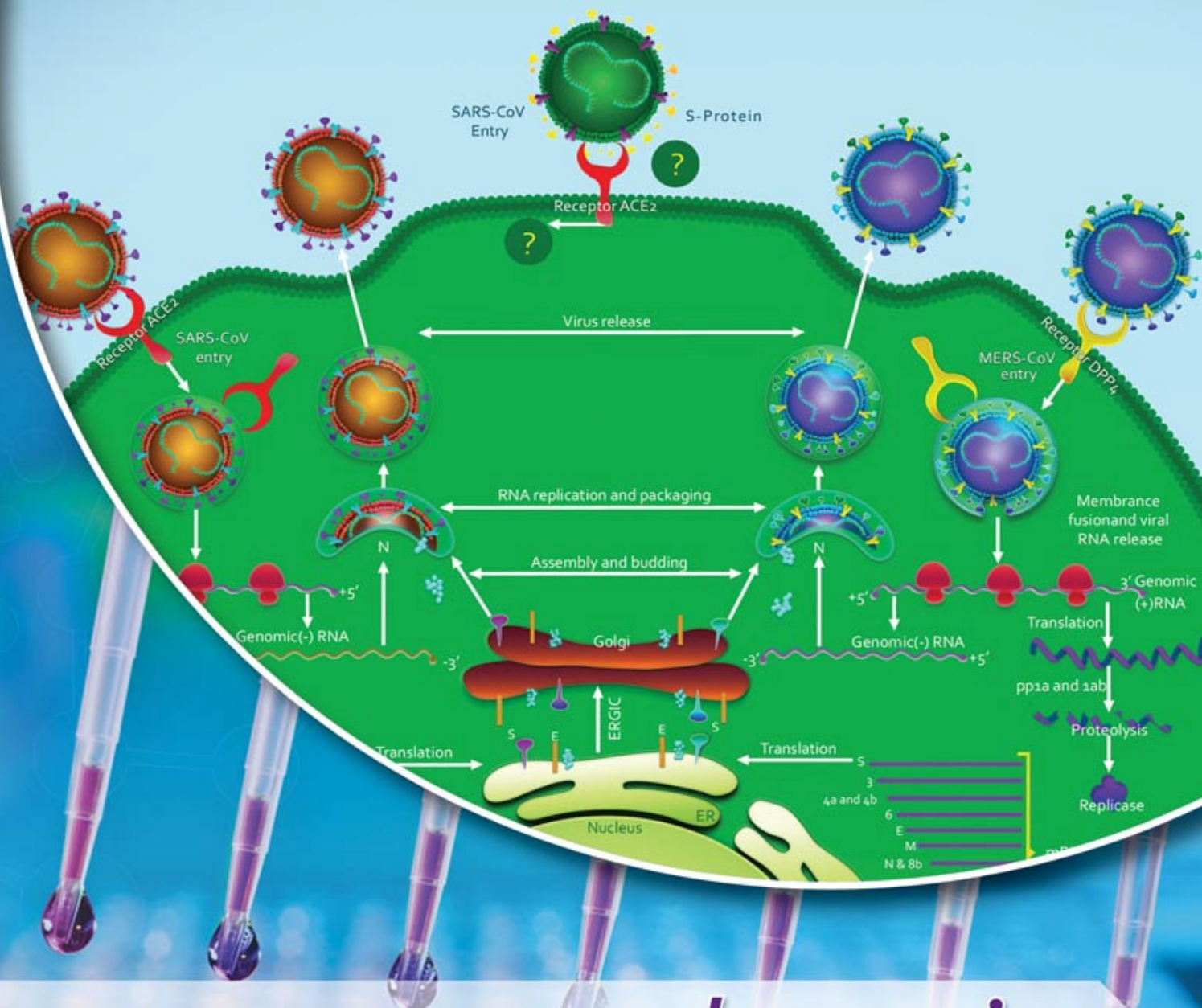


# SARS-CoV-2 Neutralizing Antibodies Detection Kit



Cat. No. 90-7005

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**Cat. No. 90-7005**

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## Table of Contents

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<b>1. Intended Use</b>	<b>3</b>
<b>2. Introduction</b>	<b>3</b>
<b>3. General References</b>	<b>3</b>
<b>4. Assay Principle</b>	<b>4</b>
<b>5. Handling and Storage</b>	<b>4</b>
<b>6. Kit Components</b>	<b>4</b>
<b>7. Materials Required (<i>but Not Supplied</i>)</b>	<b>5</b>
<b>8. General Assay Protocol</b>	<b>5-6</b>
<b>8.1. Preparation and Storage of Reagents</b>	<b>5</b>
<b>8.2. Sample Collection, Storage and Dilution</b>	<b>5</b>
<b>8.3. Assay Procedure</b>	<b>6</b>
<b>9. Typical Data</b>	<b>7</b>
<b>10. Performance Characteristics</b>	<b>7</b>
<b>11. Technical Hints, Precautions and Safety</b>	<b>8</b>
<b>12. Troubleshooting</b>	<b>9</b>

## 1. Intended Use

The SARS-CoV-2 Neutralizing Antibodies Detection Kit contains key reagents required to test the presence of functional neutralizing antibodies against SARS-CoV-2 present in the serum or plasma independently of the species and isotypes. It is an easy and fast alternative in vitro to the classical neutralization assay using Vero E6 cells. Before using this Kit to detect neutralizing antibodies against SARS-CoV-2, we recommend using Abeomic's SARS-CoV-2 (Spike RBD) IgG Serological ELISA Kit (Cat. # 90-7004) to detect the presence of IgG against SARS-CoV-2.

## 2. Introduction

Coronaviruses (CoVs) are enveloped non-segmented positive-sense single-stranded RNA viruses and can infect respiratory, gastrointestinal, hepatic and central nervous system of human and many other wild animals (1). Recently, a new severe acute respiratory syndrome  $\beta$ -coronavirus called SARS-CoV-2 (or 2019-nCoV) has emerged, which causes an epidemic of acute respiratory syndrome (called coronavirus human disease 2019 or COVID-19) (2).

SARS-CoV-2 contains 4 structural proteins, including Envelope (E), Membrane (M), Nucleocapsid (N) and Spike (S), which is a transmembrane protein, composed of two subunits S1 and S2 (3). The S1 subunit contains a receptor binding domain (RBD), which binds to the cell surface receptor Angiotensin-Converting Enzyme 2 (ACE2) present at the surface of epithelial cells, causing mainly infection of human respiratory cells (4). Following an infection with SARS-CoV-2, patients develop specific IgG and IgM immune response. A subset of these antibodies against Spike (RBD) can block virus infection / entry into cells by a process that is called *neutralization*. A strong correlation between the levels of RBD-binding antibodies and levels of SARS-CoV-2 blocking / neutralizing antibodies in patients has been observed (5).

## 3. General References

- (1) Coronaviridae. Positive Sense RNA Viruses. Available online: [https://talk.ictvonline.org/ictv-reports/ictv\\_9th\\_report/positive-sense-rna-viruses-2011/w/posrna\\_viruses/222/coronaviridae](https://talk.ictvonline.org/ictv-reports/ictv_9th_report/positive-sense-rna-viruses-2011/w/posrna_viruses/222/coronaviridae)
- (2) A pneumonia outbreak associated with a new coronavirus of probable bat origin: P. Zhou, et al.; Nature 579, 270 (2020)
- (3) The spike protein of SARS-CoV - a target for vaccine and therapeutic development: L. Du, et al.; Nat. Rev. Microbiol. 7, 226 (2009)
- (4) SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor: M. Hoffmann, et al.; Cell 181, 271 (2020)
- (5) The receptor binding domain of the viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-2 patients: L. Premkumar, et al.; Sci. Immunol. 48, eabc8413 (2020)

## 4. Assay Principle

This **SARS-CoV-2 Neutralizing Antibodies Detection Kit** is a colorimetric kit, which measures the neutralizing / blocking activity of antibodies present in human serum / plasma on the binding of the SARS-CoV-2 Spike (RBD) protein to its human receptor ACE2. SARS-CoV-2 Spike (RBD) recombinant protein has been precoated onto the 96-well microtiter plate. The serum or plasma containing antibodies against SARS-CoV-2 Spike are pipetted into the wells for binding to the coated viral protein and for blocking the interaction to ACE2. After washing to remove unbound components, ACE2 (human) protein coupled to HRP is added. Following a final washing, peroxidase activity is quantified using the substrate 3,3',5,5'-tetramethylbenzidine (TMB). The presence of neutralizing antibodies in the samples are detected by reduction of Optical Density (OD) indicating the inhibition of the Spike (RBD) - ACE2 (human) binding.

## 5. Handling and Storage

- Reagent must be stored at 2-8°C when not in use.
- The validity period is 12 months.
- Plate and reagents should reach room temperature before use.
- Do not expose reagents to temperatures greater than 25°C.

## 6. Kit Components

- 1 plate coated with Spike (RBD) (SARS-CoV-2) (6 x 16-well strips-Spike)
- ACE2 (human) (rec.) (HRP) (lyophilized) (1 vial) **(ACE2-HRP)**
- 1 vial positive Control (480 µl) **(Positive)**
- 1 vial Negative Control (480 µl) **(Negative)**
- 3 bottles Wash Buffer 10X (3 x 30 ml) **(Wash Buffer 10X)**
- 1 bottle TMB K-Blue Aqueous (12 ml) **(TMB)**
- 1 bottle Stop Solution (12 ml) **(STOP)**
- 2 plate Covers (plastic film)
- 2 silica Gel Minibags.



## 7. Materials Required (*but Not Supplied*)

- Microtiterplate reader at 450nm
- Calibrated precision pipettes. Disposable pipette tips
- Deionized water
- Microtubes or equivalent for preparing dilutions
- Disposable plastic containers for preparing working buffers
- Plate washer: automated or manual
- Glass or plastic tubes for diluting and aliquoting standard

## 8. General Assay Protocol

### 8.1. Preparation and Storage of Reagents

*NOTE: Prepare just the appropriate amount of buffers necessary for the assay.*

- **Wash Buffer 10X** has to be diluted with deionized water 1:10 before use (e.g. 30 ml Wash Buffer 10X + 270 ml water) to obtain **Wash Buffer 1X**.
- **ACE2 linked to HRP (ACE2-HRP)** has to be reconstituted with 120 µl of Wash Buffer 1X.
  - Mix the ACE2-HRP to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes **at room temperature**. Mix well prior to making dilutions.
  - After reconstitution of ACE2-HRP, prepare aliquots if needed and store them at -20°C. **Avoid freeze/thaw cycles.**
  - Dilute the reconstituted ACE2-HRP to the working concentration (e.g. 100µl in 10 ml of Wash Buffer 1X).
- **Negative Control** should be tested in duplicate. It contains diluted (1/10) human serum, negative for SARS-CoV-2 antibodies and screened for viral markers.
- **Positive Control** should be tested in duplicate. It contains a blocking anti-Spike (RBD) (SARS-CoV-2) recombinant monoclonal antibody.

### 8.2. Sample Collection, Storage and Dilution

**Serum:** Use a serum separator tube. Let samples clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1,000xg. Assay freshly prepared serum or store serum in aliquot at ≤ -20°C for later use. Avoid repeated freeze/thaw cycles.

**Plasma:** Collect plasma using heparin, citrate or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay freshly prepared plasma or store plasma sample in aliquot at ≤ -20°C for later use. Avoid repeated freeze/ thaw cycles.

**Serum and Plasma** have to be diluted in Wash Buffer 1X. Each serum / plasma sample should be tested at 1/10 as a start by mixing 25µl of serum / plasma in 250 µl of Wash Buffer 1X. If needed higher dilutions (>1/10) can be tested.

**NOTES:**

- *Diluted Serum or Plasma in Wash Buffer 1X should be used fresh.*
- *Vortex serum or plasma samples at room temperature to ensure homogeneity. Then centrifuge samples at 10,000 rpm for 5 minutes prior to assay to remove particulates. Please do not omit this centrifugation step if samples are cloudy and contain particles.*
- *Serum / Plasma from animals immunized with SARS-CoV-2 antigens can be tested using this kit.*
- *Heat inactivation at 56°C for 30 minutes is optional, but serum / plasma used should all treated the same way (either heat inactivated or not).*
- *Severe hemolytic samples should not be used.*
- *Sample safety: All samples are regarded as potentially infectious and strictly handled in accordance with relevant national standards and guidelines.*
- *This kit can measure neutralizing antibodies against SARS-CoV-2 from different isotypes and species.*

**8.3. Assay Procedure (Checklist)**

1. Determine the number of 16-well strips needed for the assay and insert them in the frame for current use. The extra strips are left in the aluminium foil bag with 2 silica gel minibags and stored at 4°C.  
*Remaining 16-well strips coated with Spike protein of SARS-CoV-2 when opened can be stored in the presence of 2 silica gel minibags at 4°C for up to 1 month.*
2. Add 100 µl of Negative control (**Negative**) and 100 µl of Positive control (**Positive**) in duplicate (**see 8.1. Preparation and Storage of Reagents**).
3. Add 100 µl of diluted (1/10) serum or plasma samples in duplicate (**see 8.1. Preparation and Storage of Reagents and 8.2 Collection, Storage and Dilution**).
4. Cover the plate with plastic film and incubate for **1 hour at 37°C**.
5. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of **five washes**. After the last wash, complete removal of liquid is essential for good performance.
6. Add 100 µl to each well of the diluted ACE2 (human)-HRP (**ACE2-HRP**) (**see 8.1. Preparation and Storage of Reagents**).
7. Cover the plate with plastic film and incubate for **1 hour at 37°C**.
8. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of **five washes**. After the last wash, complete removal of liquid is essential for good performance.
9. Add 100 µl to each well of TMB substrate solution (TMB).
10. Allow the color reaction to develop **at Room Temperature in the dark for 5-10 minutes**. Do not cover the plate.
11. Stop the reaction by adding 100 µl of Stop Solution (**STOP**). Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution (**STOP**) is added.

**! CAUTION: CORROSIVE SOLUTION !**

12. Measure the OD at 450 nm in an ELISA reader.



## 9. Typical Data

The presence of neutralizing antibodies against SARS-CoV-2 in the serum / plasma is measured by calculating the percent inhibition of each sample using the following formula:

$$= (1 - (\text{OD of the sample}) / (\text{OD of Negative Control})) \times 100$$

Samples	OD	Inhibition (%) *
Negative Control	1.77	0
Positive Control	<b>0.42</b>	<b>77.78</b>
Serum Healthy Patients	1.817	-2.13
	1.752	1.48
Serum COVID-19 Patients	<b>0.437</b>	<b>75.43</b>
	<b>1.022</b>	<b>42.55</b>
	<b>0.177</b>	<b>90.05</b>

Table 1: Examples of Optical Density (OD) and inhibition (%) of the controls and samples.

## 10. Performance Characteristics

- i) **Intra-assay:** Four serum samples were assayed in replicates 6 times to test precision within an assay.
- ii) **Inter-assay:** Three serum samples were assayed in 4 separate assays to test precision between assays.

Intra-assay (n=6)	
Samples	CV (%)
1	3.72
2	7.94
3	8.26
4	4.55
Inter-assay	
Samples	CV (%)
1	7.11
2	11.37
3	7.85

Table 2: Examples of intra and inter-assays of different samples.

- iii) **Cutoff suggestion:** With the panels of samples tested, we can propose the following cutoff values for the presence of neutralizing antibodies in the serum / plasma of COVID-19 positive patients:
  - For samples without neutralizing antibodies, inhibition (%) should be < 20%.
  - For samples with neutralizing antibodies, inhibition (%) should be > 20%.

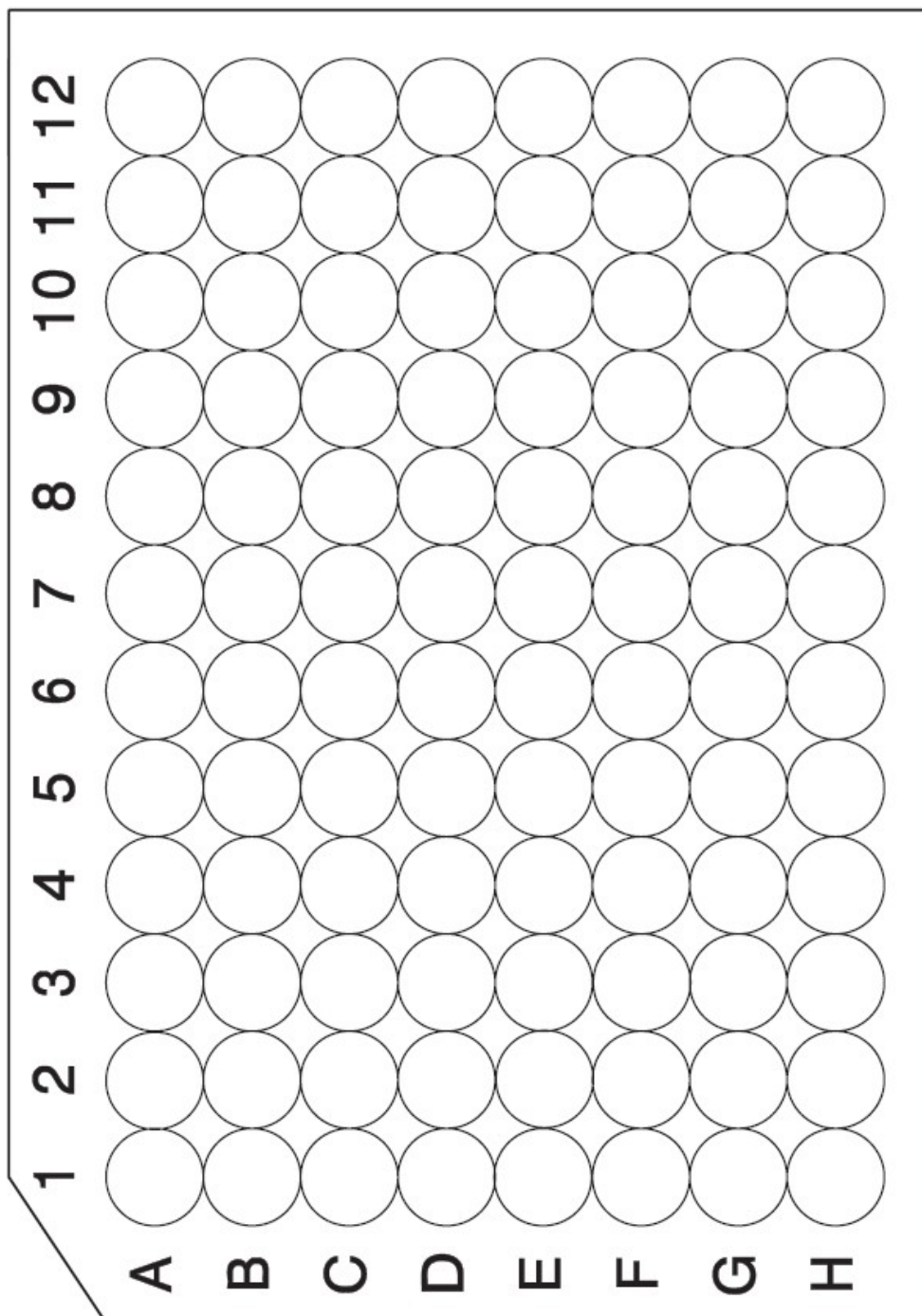
## 11. Technical Hints, Precautions and Safety

- It is recommended that samples to be run in duplicate.
  - Do not combine leftover reagents with those reserved for additional wells.
  - The kit should not be used beyond the expiration date on the kit label.
  - Reagents from the kit with a volume less than 100 µl should be centrifuged.
  - Residual wash liquid should be drained from the wells after last wash by tapping the plate on absorbent paper.
  - Crystals could appear in the 5X or 10X solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solution.
  - Once reagents have been added to the 16-well strips, DO NOT let the strips DRY at any time during the assay.
  - Keep TMB Solution protected from light.
  - When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.
  - The Stop Solution (STOP) consists of sulfuric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.
  - All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.
  - This test is only for qualitative detection.
  - Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.
- If the result for neutralizing antibodies is negative while the patient was confirmed positive for COVID-19 by PCR, it is recommended to collect a new sample from the patient a few days later and test it again.

## 12. Troubleshooting

PROBLEM	POSSIBLE CAUSES	SOLUTIONS
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be followed as indicated in the manual.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of HRP too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.

## Plate Layout Template



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