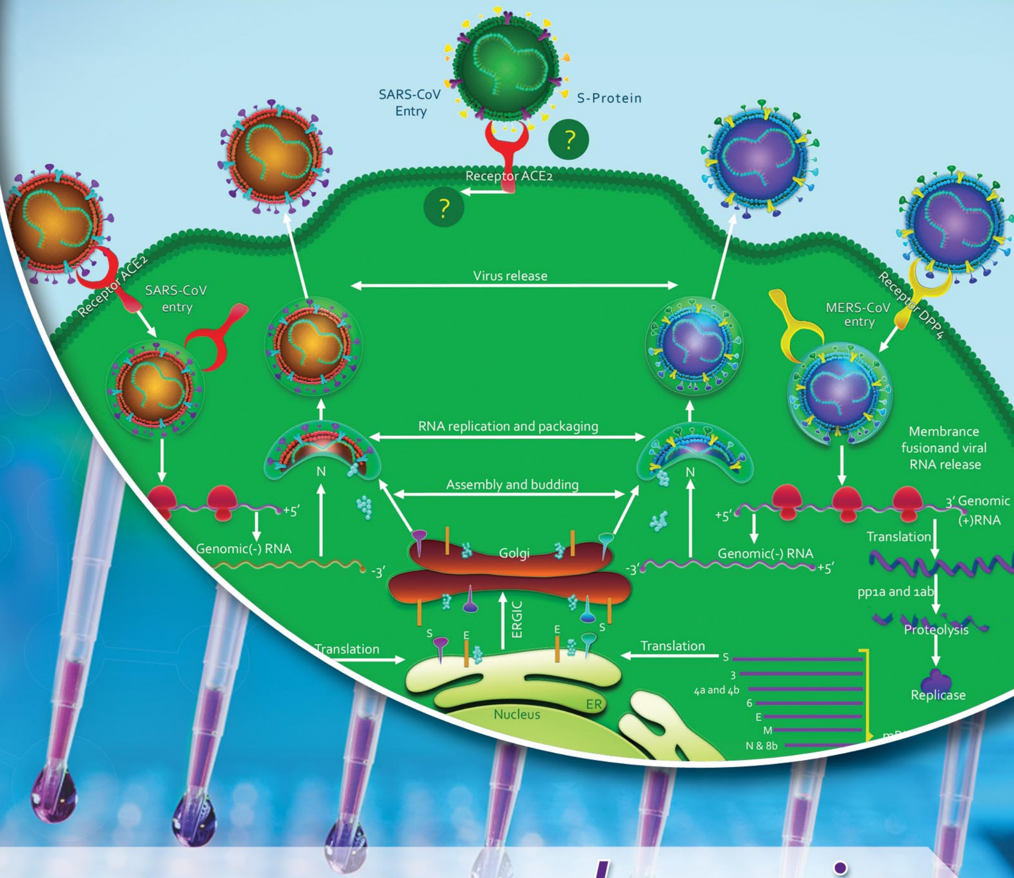
Covid-19 Nucleoprotein Pre-Coated ELISA Kit



Cat.No: 90-7006

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Covid-19 Nucleoprotein Pre-coated ELISA Kit

Catalog No: 90-7006
1 × 96 well Format (96 tests)
Detection Range: 62.5-4000pg/ml
Sensitivity: <37.5pg/ml

In vitro quantitative determination of COVID-19 nucleoprotein concentrations in serum, plasma, cell culture supernatant and other biological samples.

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I. BACKGROUND

2019-nCoV (2019-NCoV) is a new coronavirus, which belongs to β-CoV of the reticulariviridaecoroni viridae with SARS-CoV. It is an unsegmented single-stranded positive RNA virus with a genome length of about 30,000 nucleotides per group. In terms of gene sequence homology, the 2019-nCoV genome has 80% similarity with SARS, and the 2019-nCoV gene sequence has 40% similarity with MERS-CoV gene sequence. S protein is the most important membrane protein on the surface of coronavirus. Studies have shown that the S protein is closely related to the process of coronavirus invasion into cells. N protein is another important structural protein in coronaviruses. In the coronavirus particle, the N protein is at the core of the virus particle and exists in the form of Genomic RNA.

II. OVERVIEW

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Anti COVID-19 nucleoprotein antibody (mouse monoclonal Ab) was pre-coated onto the 96-well plate. The biotin conjugated anti COVID-19 nucleoprotein antibody (mouse monoclonal Ab) was used as the detection antibody. The standards and pilot samples were added to the wells subsequently. After incubation, unbound conjugates were removed by wash buffer. Then, biotinylated detection antibody was added to bind with COVID-19 nucleoprotein conjugated on coated antibody. After washing off unbound conjugates, HRP-Streptavidin was added. After a third washing, TMB substrates were added to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that turned yellow after adding a stop solution. Read the O.D. absorbance at 450nm in a microplate reader. The concentration of COVID-19 nucleoprotein in the sample was calculated by drawing a standard curve. The concentration of the target substance is proportional to the OD450 value.

III. ADVANTAGES

Multiple samples can be analyzed in a low volume, high-throughput format. Full analysis can be completed in 2 hours.

IV. REQUIRED INSTRUMENTS AND REAGENTS

1. Microplate reader (wavelength: 450nm)
2. 37°C incubator (CO_2 incubator for cell culture is not recommended.)
3. Automated plate washer or multi-channel pipette/5ml pipettor (for manual washing purpose)
4. Precision single (0.5-10μL, 5-50μL, 20-200μL, 200-1000μL) and multi-channel pipette with disposable tips (calibration is required before use.)
5. Sterile tubes and Eppendorf tubes with disposable tips
6. Absorbent paper and loading slot
7. Deionized or distilled water.

V. SAMPLE COLLECTION AND STORAGE

The following sample processing steps are concise operations.

1. Serum

Place whole blood sample at room temperature for 2 hours or at 2-8°C overnight. Centrifuge for 20min at 1000xg and collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay.

2. Plasma

EDTA-Na2/K2 is recommended as the anticoagulant. Centrifuge samples for 15 minutes at 1000×g 2-8°C within 30 minutes after collection. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay. For other anticoagulant types and uses, please refer to the sample preparation guideline.

3. Tissue Sample

Generally tissue samples are required to be made into homogenization. Protocol is as below:

3.1. Place the target tissue on the ice. Remove residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Then weigh for usage.

3.2. Use lysate to grind tissue homogenates on the ice. The adding volume of lysate depends on the weight of the tissue. Usually, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS (e.g. 1mM PMSF).

3.3. Do further process using ultrasonic disruption or freeze-thaw cycles (Ice bath for cooling is required during ultrasonic disruption; Freeze-thaw cycles can be repeated twice.) to get the homogenates.

3.4. Homogenates are then centrifuged for 5 minutes at 5000×g. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay.

3.5. Determine total protein concentration by BCA kit for further data analysis. Usually, total protein concentration for Elisa assay should be within 1-3mg/ml. Some tissue samples such as liver, kidney, pancreas which containing a higher endogenous peroxidase concentration may react with TMB substrate causing false positivity. In that case, try to use 1% H_2O_2 for 15min inactivation and perform the assay again.

Notes: PBS buffer or the mild RIPA lysis can be used as lysates. While using RIPA lysis, make the PH=7.3. Avoid using any reagents containing NP-40 lysis buffer, Triton X-100 surfactant, or DTT due to their severe inhibition for kits' working. We recommend using 50mM Tris+0.9%NaCL+0.1%SDS, PH7.3. You can prepare by yourself or contact us for purchasing.

VI. KIT COMPONENTS

Item	Specifications	Storage
96 well Strip ELISA Plate	8 X 12 well	4°C
Lyophilized Standard	2 vials	-20°C
Sample and Standard Dilution Buffer	20 ml	4°C
Biotinylated Detection Antibody	120 µl	4°C in dark
Antibody Dilution Buffer	10 ml	4°C
HRP Conjugated Streptavidin	120 µl	4°C in dark
SABC Dilution Buffer	10 ml	4°C
TMB Substrate	10 ml	4°C in dark
Stop Solution	10 ml	4°C
25X Wash Buffer	30 ml	4°C
Plate Sealer	5 pieces	
Product Manual	1 copy	

VII. PRECAUTIONS FOR USE

1. When using different Elisa kits, labeling is required to avoid mixed components and failed assay.

2. After opening the kit, please refer to the table of storage condition for coated plate and standards (Dampness may decrease the activity). If any component is missing or damaged during the assay or storage, please contact us for ordering a new one to replace (e.g. E002 lyophilized standard).

3. Sterile and disposable tips are required during the assay. After use, the reagents bottle cap has to be tightened to avoid the microbial contamination and evaporation.

4. While manual washing, please keep tips or pipettors for adding wash buffer away from the well. Insufficient washing or contamination easily causes false positive and high background.

5. During the assay, prepare required reagents for next step in advance. After washing, add the reagent into the well in time to avoid dryness. Otherwise, dry plate will result in the failed assay.

6. Before confirmation, reagents from other batches or sources should not be used in this kit.

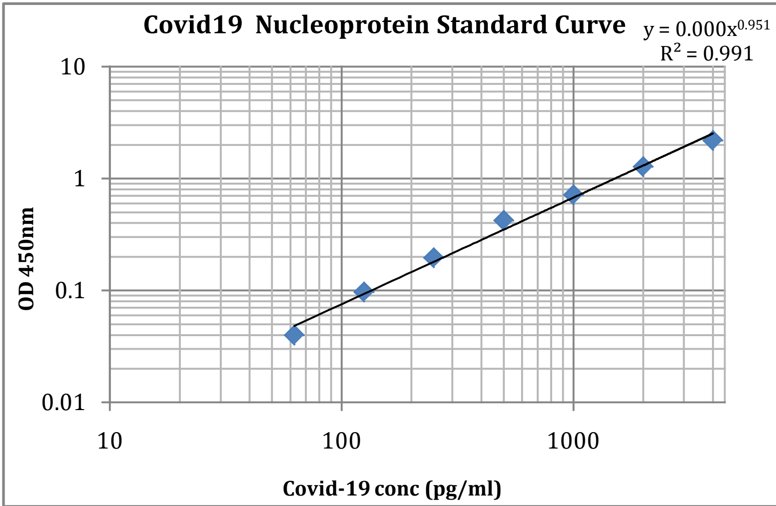
7. Don't reuse tips and tubes to avoid cross contamination.

8. After loading, seal the plate to avoid the evaporation of the sample during incubation. Complete the incubation process at recommended temperature.

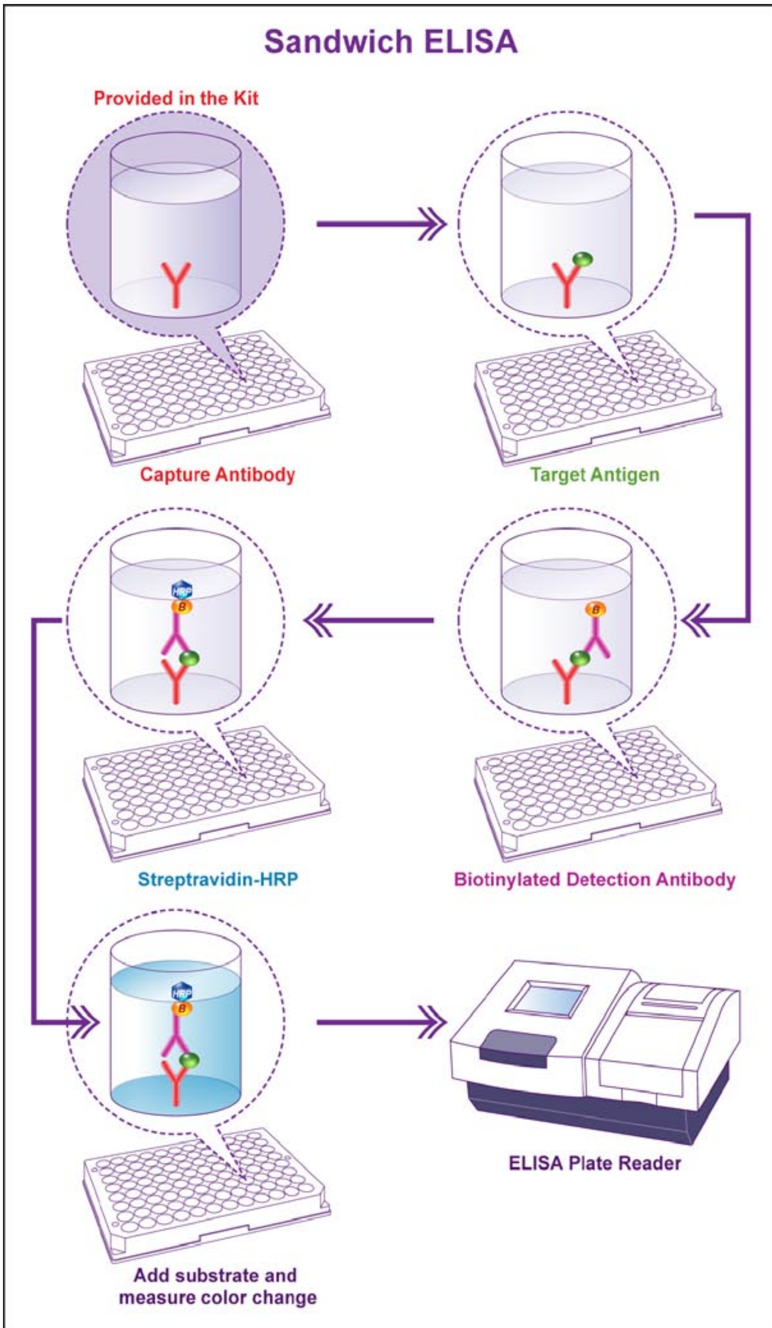
9. Please wear the lab coat, mask and gloves to protect yourself during the assay. Especially, for the detection of blood or other body fluid samples, please follow regulations on safety protection of biological laboratory.

VIII. STANDARD CURVE

Covid 19 Nucleoprotein Standard Curve is shown below.



X	pg/ml	4000	2000	1000	500	250	125	62.5	0
Y	O.D.450	2.318	1.405	0.845	0.549	0.322	0.223	0.166	0.123


Sandwich ELISA
Provided in the Kit
Capture Antibody
Target Antigen
Streptravidin-HRP
Biotinylated Detection Antibody
Add substrate and
measure color change
ELISA Plate Reader

IX. REAGENT PREPARATION AND STORAGE

Included buffers and reagents are optimized for use with this kit. Substitution with other reagents is not recommended and may not give optimal results.

1. **Prepare Standard Curve:** One hour before the experiment. Use one tube for each experiment.

   a. Quick spin down one vial of lyophilized standard. ***(DO NOT dilute standard directly on the plate)***. Add 1ml of sample/standard dilution buffer into one of the standard tube. Incubate at room temp. for 10 min. Mix thoroughly by vortex. Stock Standard concentration is 8000 pg/ml.

   Note: *If the standard tube concentration higher than the range of the kit, please dilute it and labeled as zero tube.*

   b. Label 6 eppendorf tubes with 4000pg/ml, 2000pg/ml, 1000pg/ml, 500 pg/ml, 250pg/ml, 125pg/ml respectively. Add 0.3 ml of sample/ standard dilution buffer into each tube. Add 0.3 ml of stock standard (8000pg/ml) into 1st tube and mix thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube mix thoroughly, and so on.

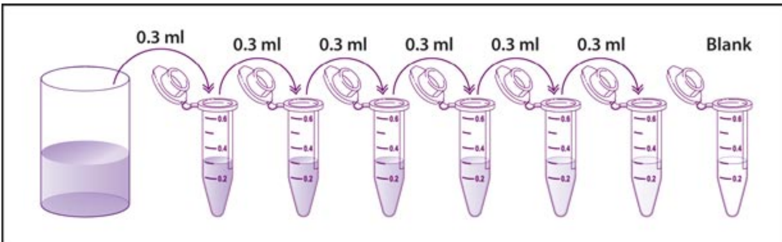


Fig-1: Dilution tubes

Note: *Standard Solutions are best used within 2 hrs. Standard solution should be stored at 4°C for up to 12 hrs. or store at -20°C for up to 48 hrs. Avoid repeated freeze-thaw.*

2. **Sample preparation and storage:** Test samples should be collected, analyze immediately (within 2 hrs.) or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

a. **Cell culture supernatants**: Centrifuge to remove precipitate, analyze immediately or aliquot and store at -20°C.

b. **Serum**: Coagulate the serum at room temp about 1 hr. Centrifuge approximately 1000 × g for 15 min. Analyze serum immediately or aliquot and store at -20°C.

c. **Plasma:** Collect plasma with heparin or EDTA as the anti-coagulant. Centrifuge for 15 min at 2-8°C at 1500 × g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10,000 × g. Analyze immediately or aliquot and store frozen at -20°C.

d. **Tissue Homogenates**: For general information, hemolytic blood may affect the results, you should rinse the tissues with ice cold PBS (0.01M, pH 7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces. This will be homogenized in PBS in a cold glass homogenizer. *(Volume depends on the weight of the tissue, 1gram of tissue requires 9 ml of ice cold PBS with protease inhibitor)*. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze- thaw cycle. Homogenates are then centrifuged for 5 min. at 5000 × g to get the supernatant.

Note: *Samples to be used within 5 days may be store at 4°C, otherwise sample should be stored at -20°C (<1 month) or -80°C (<2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this Assay.*

e. End user should estimate the concentration of the target protein in the test samples first, then select proper dilution factor to make the diluted target protein concentration falls the optimal detection range of the kit. Dilute the samples with the provided dilution buffer. Several trials may be necessary in practice. The test sample should be well mixed with the dilution buffer. Standard curve and sample should be made before the experiment.

High target protein concentration (10000-100000 pg/ml) : Dilute 1:100 (add 1 µl of sample into 99 µl of sample/ standard dilution buffer).

Medium target protein concentration (1000-10000 pg/ml) : Dilute 1:10 (i.e. add 10 µl of sample into 90 µl of sample/ standard dilution buffer).

Low target protein concentration (15.6-1000 pg/ml): Dilute 1:2 (i.e. add 50 µl of sample into 50 µl of sample/ standard dilution buffer).

Very low target protein concentration (≤15.6 pg/ml): Unnecessary to dilute, or dilute at 1:2.

3. **Preparation of Biotin detection antibody working solution:** Prepare within one hour before the experiment. Calculate total volume working solution required. (0.1 ml/well × number of wells. Add 100-200 µl extra).

   Dilute Biotin detection antibody with antibody dilution buffer at 1:100 and mix thoroughly. (*i.e.* add 1 µl of Biotin conjugated detection antibody into 99 µl of antibody dilution buffer).

4. **Preparation of HRP-Streptavidin Conjugate (SABC) working solution:** Prepare within 30 min before the experiment. Calculate total volume working solution required. (0.1 ml/well × number of wells. Add 100-200 µl extra).

   Dilute SABC with SABC dilution buffer at 1:100 and mix thoroughly. (*i.e.* add 1 µl of SABC into 99 µl of SABC dilution buffer).

5. **Preparation of 1 X Wash buffer:**

   If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely been dissolved. The solution should be cooled to room temperature before use.

   Dilute 30ml (15ml for 48T) Concentrated Wash Buffer into 750ml (375ml for 48T) Wash Buffer with deionized or distilled water. Put unused solution back at 2-8°C.

Prepare 1 X Wash buffer by diluting 25X Wash buffer in sterile water. Diluted wash buffer may be stored at 4°C, however we recommend preparing fresh 1X wash buffer for each experiment.

For example: 10 ml of 25X Wash buffer in 240 ml of sterile water.

X. ASSAY PROCEDURE

Before starting the experiment, equilibrate the SABC working solution and TMB substrate for at least 30 min at room temp. When diluting samples and reagents, they should be mixed completely and evenly. It is recommended to plot a standard curve for each test.

** If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.*

1. Set standard, test sample and blank (control zero) wells on the pre-coated plate and then record their position. It is recommended to measure each standard and sample in duplicate.

   Note: *Wash plate twice before adding standard, sample and blank into the well.*

2. Add 0.1 ml of standard (4000pg/ml, 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, control zero dilution buffer) into standard well.
3. Add 0.1 ml of diluted samples into test sample wells.
4. Seal plate with a cover and incubate at 37°C for 90 min.
5. Remove the cover and discard samples and standard solution by tapping plate on an absorbent paper. ***Note:*** *DO NOT let the wells completely dry any time. DO NOT wash plate.*
6. Add 0.1 ml of Biotin-detection antibody working solution into the above wells (Standards, control zero and samples).
7. Seal plate with cover and incubate at 37°C for 60 min.
8. Remove the cover, and wash plate 3 times with 1X wash buffer.
9. Add 0.1 ml of SABC working solution into each well. Cover the plate and incubate at 37°C for 30 min.

10. Remove the cover and wash plate 5 times with 1X wash buffer. Each time let the wash buffer stay in the well for 1-2 min.
11. Add 90 µl of TMB substrate into each well, cover the plate and incubate at 37°C in dark within 15-30 min. *(**Note**: This incubation time is for reference use only. The optimal time should be determined by end user)*. The shades of blue can be seen in the first 3-4 wells, only on most concentrated standards. Other wells show no obvious color.
12. Add 50 µl of stop solution into each well and mix thoroughly. Color will change into yellow immediately.
13. Read O.D. absorbance at 450 nm in a micro-plate reader immediately after adding the stop solution.
14. Calculation: Relative O.D. 450 = O.D. for each well – O.D. 450 control zero well. The standard curve can be plotted as the relative O.D. 450 of each standard solution in Y axis vs. the respective concentration of the standard in X axis. Concentration of the samples can be incorporated from the standard curve. If the samples were diluted, multiply the dilution factor to the concentration.

Table-1

	Standard1	Standard2	3	4	5	6	7	8	9	10	11	12
A	4000pg/ml	4000pg/ml										
B	2000pg/ml	2000pg/ml										
C	1000pg/ml	1000pg/ml										
D	500pg/ml	500pg/ml										
E	250pg/ml	250pg/ml										
F	125pg/ml	125pg/ml										
G	62.5pg/ml	62.5pg/ml										
H	0	0										

XI. TROUBLE SHOOTING

Problem	Probable Cause	Suggestion
No signal	Forgot to add all components.	Prepare check list and add the components in the correct order.
Low signal	Not enough lysates per well.	Check the protein concentration. Add more lysates.
High background	Washing is not sufficient.	Wash plates thoroughly after incubation with Streptavidin-HRP secondary

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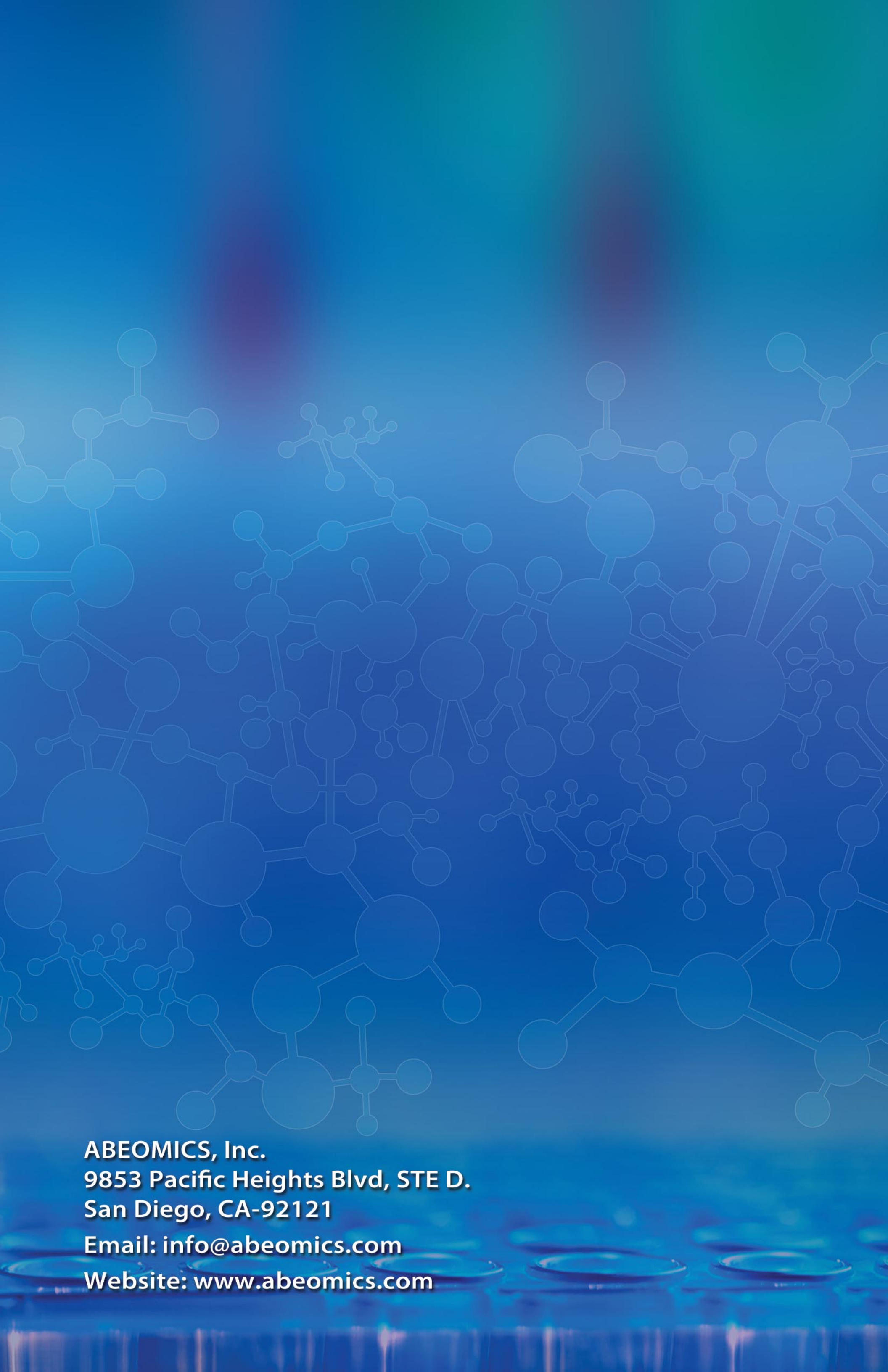
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