SARS-CoV-2 (2019-nCoV) Nucleoprotein / NP ELISA Kit

Catalog Number: KIT40588

Please read this instruction manual carefully before using the product.

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BACKGROUND

Coronaviruses are enveloped viruses with a positive-sense RNA genome and with a nucleocapsid of helical symmetry. Coronavirus nucleoproteins localize to the cytoplasm and the nucleolus, a subnuclear structure, in both virus-infected primary cells and in cells transfected with plasmids that express N protein. Coronavirus N protein is required for coronavirus RNA synthesis, and has RNA chaperone activity that may be involved in template switch. Nucleocapsid protein is a most abundant protein of coronavirus. During virion assembly, N protein binds to viral RNA and leads to formation of the helical nucleocapsid. Nucleocapsid protein is a highly immunogenic phosphoprotein also implicated in viral genome replication and in modulating cell signaling pathways. Because of the conservation of N protein sequence and its strong immunogenicity, the N protein of coronavirus is chosen as a diagnostic tool.

INTENDED USE

The kit has been verified by high purity SARS-CoV-2 (2019-nCoV) Nucleoprotein / NP Protein (Cat# 40588-V08B). It can recognize recombinant SARS-CoV Nucleoprotein / NP Protein (His Tag) (Cat# 40143-V08B), but no react with recombinant MERS-CoV Nucleoprotein / NP protein (His Tag) (Cat# 40068-V08B).

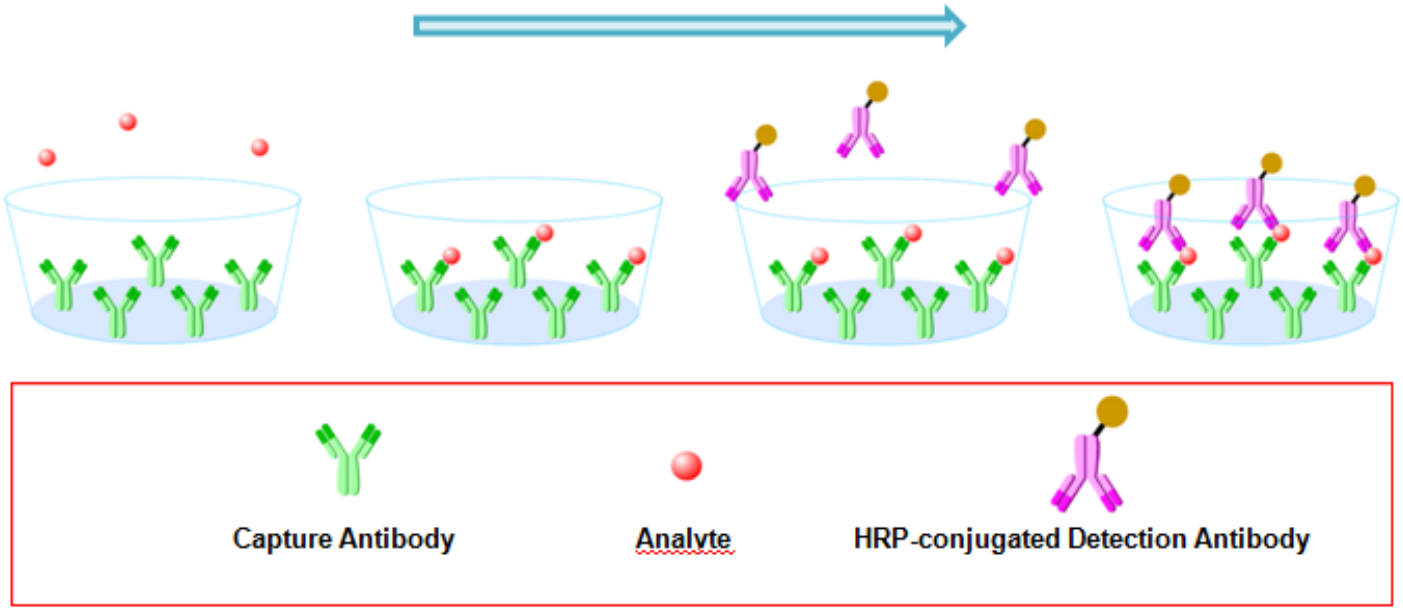
The use of this kit for natural samples need be validated by the end user due to the complexity of natural targets and unpredictable interference.

ALTERNATIVE NAMES

SARS-CoV-2, 2019-nCoV, COVID-19, NCP, Nucleoprotein, NP

PRINCIPLE OF THE ASSAY

The principle of this ELISA kit is based on the solid phase sandwich enzyme immunoassay technique. A monoclonal antibody specific for SARS-CoV-2 (2019-nCoV) Nucleoprotein / NP has been pre-coated onto well plate strips. Standards and samples are added to the wells and SARS-CoV-2 (2019-nCoV) Nucleoprotein / NP present in the sample is bound by the immobilized antibody. After incubation the wells are washed and a horseradish peroxidase conjugated anti-SARS-CoV-2 (2019-nCoV) Nucleoprotein / NP antibody is added, producing an antibody-antigen-antibody "sandwich complex". Following a wash to remove any unbound antibody a TMB substrate solution is loaded and color develops in proportion to the amount of SARS-CoV-2 (2019-nCoV) Nucleoprotein / NP bound. The reaction is stopped by the addition of a stop solution and the intensity of the color can be measured at 450 nm (See schematics below).



MATERIALS PROVIDED

SARS-CoV-2 (2019-nCoV) Nucleoprotein / NP Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with rabbit mAb antibody against SARS-CoV-2 (2019-nCoV) Nucleoprotein / NP.

SARS-CoV-2 (2019-nCoV) Nucleoprotein / NP Detection Antibody - 0.2 mg/mL of rabbit mAb antibody against SARS-CoV-2 (2019-nCoV) Nucleoprotein / NP conjugated to horseradish peroxidase (HRP) with preservatives.

SARS-CoV-2 (2019-nCoV) Nucleoprotein / NP Standard - Recombinant SARS-CoV-2 (2019-nCoV) Nucleoprotein / NP in a buffer with preservatives, lyophilized. The amount of standard is lot specific and indicated on the label of standard vial.

Wash Buffer Concentrate - 25 mL of a 20-fold concentrated solution of buffered surfactant with preservatives.

Dilution Buffer Concentrate - 8 mL of a 20-fold concentrated dilution buffer with preservatives.

Color Reagent A - 13 mL of stabilized hydrogen peroxide.

Color Reagent B - 13 mL of stabilized chromogen (tetramethylbenzidine).

Stop Solution - 8 mL of 2 N sulfuric acid.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8℃ and the kit is stable for 6 months upon receipt.</td></tr>
<tr><td rowspan="7">Opened/ Reconstituted Reagents</td><td>Diluted Wash Buffer</td><td rowspan="2">Stored for up to 1 week at 2 - 8℃</td></tr>
<tr><td>Diluted Dilution Buffer</td></tr>
<tr><td>Conjugate</td><td rowspan="4">Stored for up to 1 month at 2 - 8℃</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td></tr>
<tr><td>Standard</td><td>After reconstitution, store for up to 1 month at -80℃.
The reconstituted standards should be aliquoted and
avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused strips to the foil pouch containing the desiccant pack and reseal along entire edge of zip-seal.
Stored for up to 1 month at 2 - 8℃</td></tr>
</table>

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Multi -channel pipette, squirt bottle, manifold dispenser, or automated microplate washer
- 500 mL graduated cylinder
- Tubes for standard dilution
- Well plate cover or seals

PRECAUTIONS

1. This kit is **for research use only** and is not for use in diagnostic or therapeutic procedures.

2. The kit should not be used beyond the expiration date.

3. Do not mix reagents from different lots.

4. The kit is designed and tested to detect the specific targets and samples shown in the manual. The use of this kit for other purpose should be verified carefully by the end user.

SAFETY INSTRUCTIONS

5. The Stop Solution provided with this kit is an acid solution. Take care when using the reagent to avoid the risk.

6. All biological materials should be handled and discarded as potentially hazardous following local laws and regulations.

7. Personal protective equipments such as lab coats, gloves, surgical masks and goggles are necessary in experiments for safety reasons.

TECHINICAL TIPS

8. Bring all reagents and samples to room temperature before use.

9. Samples should be thawed completely and mixed well prior to analysis. Avoid repeated freeze-thaw cycles of frozen samples.

10. A standard curve should be generated for each set of sample assayed. DO NOT USE the standard curves from other plates or other days.

11. Use a new disposable reagent reservoir and new disposable pipette tips for each transfer to avoid cross-contamination.

12. Read the absorbance of each well within 20 minutes after adding the stop solution.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20℃ or lower temperature. **Avoid repeated freeze -thaw cycles.**

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20℃ or lower temperature. **Avoid repeated freeze-thaw cycles. If the use of original supernate sample or low dilution (<5 fold) are necessary due to the expected low concentration of antigen supernates need be adjust to neutral pH condition before assay.**

Note:

The sample should be diluted to within the working range of the assay in 1× dilution buffer. The exact dilution must be determined based on the concentration of specific target in individual samples.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

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

sections.

2. Remove unused microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

3. Wash each well three times with Wash Buffer (300 µL/well) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. **Complete removal of liquid at each step is essential to good performance. Remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.**

4. Add 100 µL of each serially diluted protein standard or test sample per well including a zero standard. **Ensure reagent addition is uninterrupted and completed within 15 minutes.** Cover/seal the plate and incubate for 2 hours at room temperature.

5. Repeat the aspiration/wash as in Step 3.

6. Add 100 µL of Detection Antibody in working concentration to each well. Cover/seal the plate and incubate for 1 hour at room temperature.

7. Repeat the aspiration/wash as in Step 3.

8. Add 200 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature**. Protect from light.**

9. Add 50 µL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

10. **Determine the optical density of each well within 20 minutes**, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

If samples generate values higher than the highest standard, dilute the samples and repeat the assay.

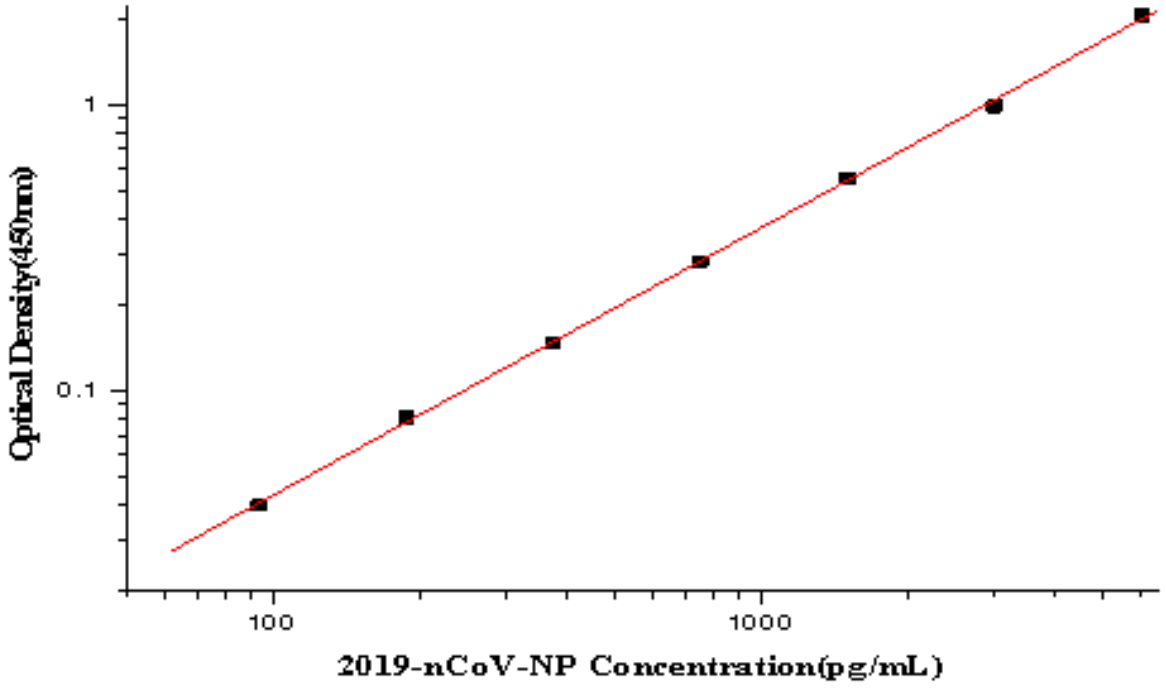
Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (O.D.) .

Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. Most graphing software can help make the curve and a four parameter logistic (4-PL) usually provide the best fit, though other equations (e.g. linear, log/log) can also be tried to see which provides the most accurate. Extrapolate the target protein concentrations for unknown samples from the standard curve plotted.

TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.

Concentration (pg/mL)	Zero standard subtracted OD
0	0
93.75	0.040
187.5	0.081
375	0.147
750	0.285
1500	0.557
3000	0.989
6000	2.046



RECOVERY

The recovery of SARS-CoV-2 (2019-nCoV) Nucleoprotein / NP spiked to different levels throughout the range of the assay in related matrices was evaluated.

Sample	Average % Recovery	Range
Serum (n=3)	81	79 -83%
Cell culture supernates (n=3)	117	111 -127%

LINEARITY

		Serum	Cell culture supernates
1:2	recovery of detected	80%	101%
1:4	recovery of detected	92%	102%
1:8	recovery of detected	108%	97%
1:16	recovery of detected	126%	93%

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant SARS-CoV-2 (2019-nCoV) Nucleoprotein / NP produced at Sino Biological Inc., (Cat# 40588-V08B).

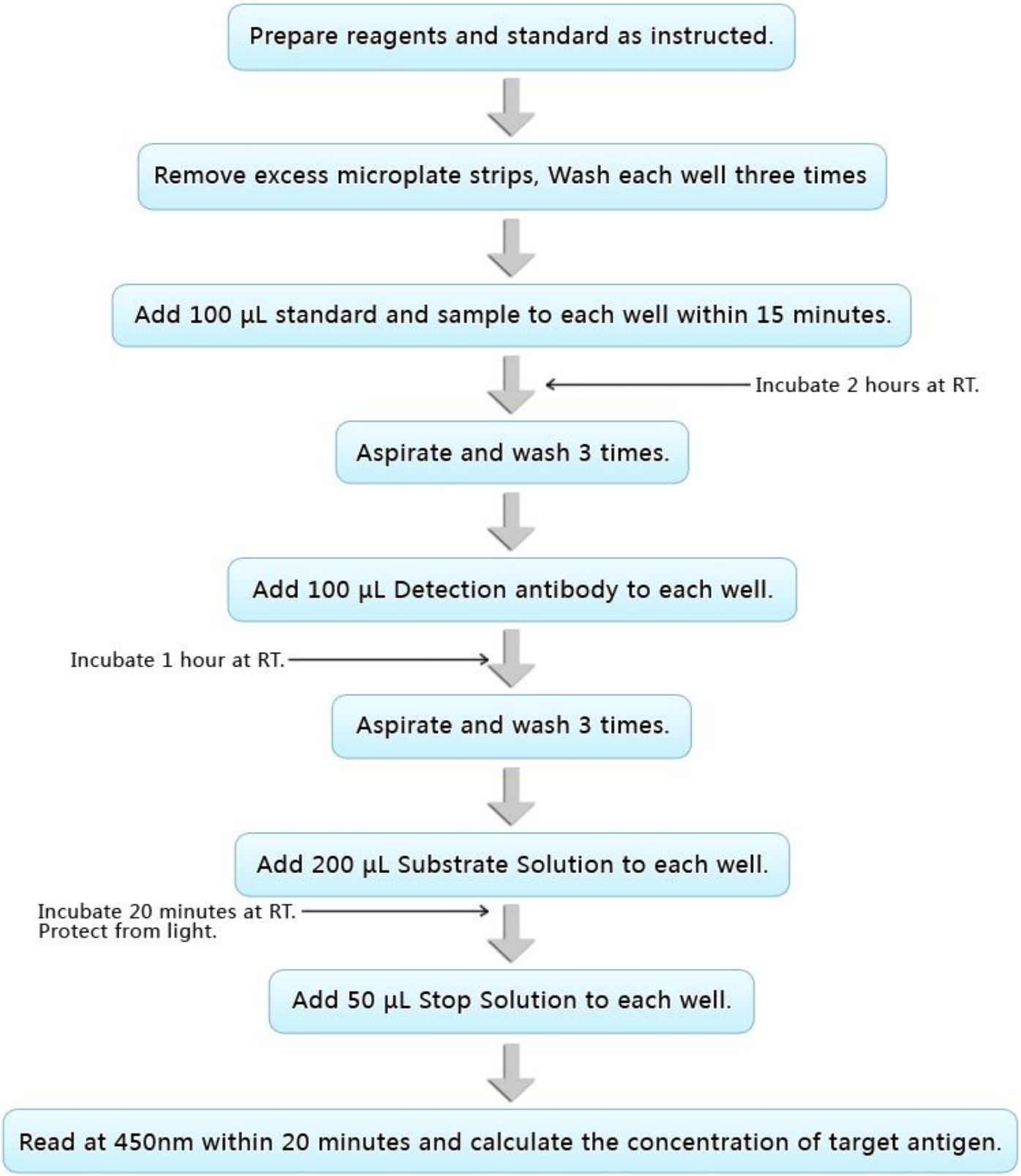
SPECIFICITY

It can recognize recombinant SARS-CoV-2 (2019-nCoV) Nucleoprotein / NP Protein (Cat# 40588-V08B), and SARS-CoV Nucleoprotein / NP Protein (His Tag) (Cat# 40143-V08B), but no react with recombinant MERS-CoV Nucleoprotein / NP protein (His Tag) (Cat# 40068-V08B).

TROUBLE SHOOTING

Problems	Possible Sources	Solutions
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Standard was incompletely reconstituted or was inappropriately stored	Aliquot reconstituted standard and store at -80 ℃. The reconstituted standards should be aliquoted and avoid repeated freeze-thaw cycles.
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature, timing or agitation	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	Color Reagent should be clear and colorless prior to addition to wells	Color Reagent should be clear and colorless prior to addition to wells
	Use clean tubes and pipettes tips	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples

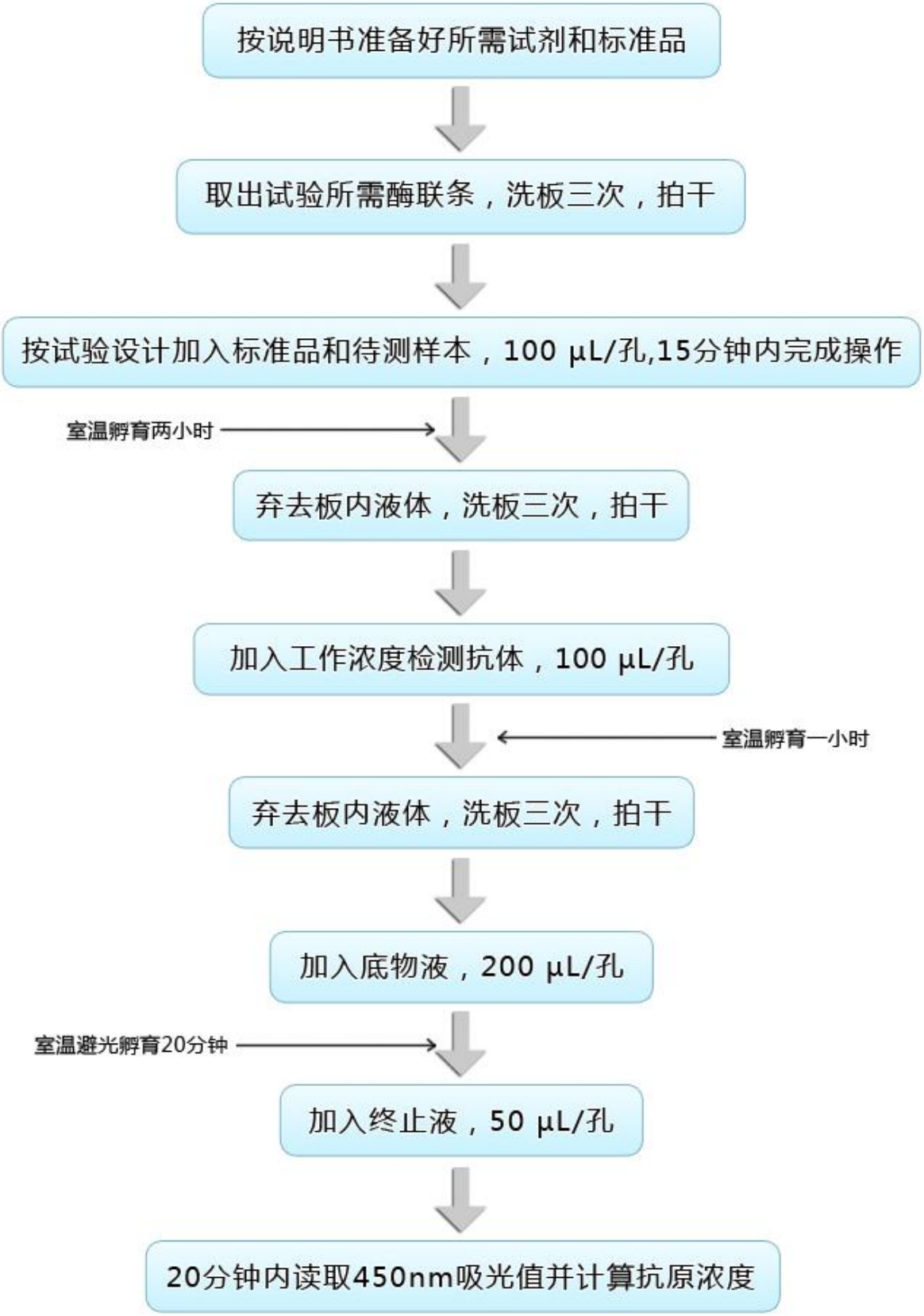
ASSAY SUMMARY



http://doc.sinobiological.com/reagent/ASSAY_SUMMARY.pdf

ASSAY SUMMARY_中文版

实验流程汇总简图



http://doc.sinobiological.com/reagent/ASSAY_SUMMARY_Chinese_version.pdf

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