GENUIN® COV2 ENRICHMENT KIT #VKN1

CATALOG & PRICING

Cat. #	Size
VKN1-10	1x 10 Runs
VKN1-50	5x 10 Runs

INTENDED USE

Accurate detection of SARS-CoV-2 is difficult, particularly when viral levels are low. First, no tests, whether RT-PCR or immunoassay, are 100% accurate; consequently, false negative or positive results are unavoidable (Shuren and Stenzel, 2020). False negative outcomes will increase the risk of viral spread, while false positive outcomes will increase the burden on healthcare providers and unnecessary quarantine (Shuren and Stenzel, 2020). Second, the virial load of various types of clinical specimens can vary considerably. Clinical studies indicate that lower respiratory tract specimens tend to have a higher viral load (Hu et al., 2020; Wang et al., 2020). Specifically, the bronchoalveolar lavage fluid specimens contain the highest vial load, followed by sputum, nasal swabs, fibrobronchoscope brush biopsy, pharyngeal swabs, feces, and blood (Wang et al., 2020). Finally, the presence of SARS-CoV-2 in water and wastewater transmitted from patients' feces and masks has raised significant environmental concerns (Tran et al., 2020). However, it is technically challenging to detect SARS-CoV-2 at a very low level.

One way to increase the sensitivity and accuracy of SARS-CoV-2 detection is to enrich the coronaviruses from biospecimens or samples. To this end, we developed the GenuIN® CoV2 Enrichment Kit to concentrate SARS-CoV-2 nucleocapsid proteins in solutions with magnetic beads. Enriched N proteins can be used for subsequent applications such as immunoprecipitation, ELISA, lateral flow immunoassay, and proteomic research.

Hu, B., Guo, H., Zhou, P., and Shi, Z.L. (2020). Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol.
Shuren, J., and Stenzel, T. (2020). Covid-19 Molecular Diagnostic Testing - Lessons Learned. N Engl J Med 383, e97.
Tran, H.N., Le, G.T., Nguyen, D.T., Juang, R.S., Rinklebe, J., Bhatnagar, A., Lima, E.C., Iqbal, H.M.N., Sarmah, A.K., and Chao, H.P. (2020). SARS-CoV-2 coronavirus in water and wastewater: A critical review about presence and concern. Environ Res, 110265.
Wang, W., Xu, Y., Gao, R., Lu, R., Han, K., Wu, G., and Tan, W. (2020). Detection of SARS-CoV-2 in Different Types of Clinical Specimens. JAMA 323, 1843-1844.

PRODUCT FEATURES

- Reay-to-use kit including required components
- Detailed instructions are simple to understand and follow
- Suitable to enrich SARS-CoV-2 at very low levels

PRODUCT DETAILS

1) COMPONENTS INCLUDED WITH THE TEST:

Components manufactured by GenuIN® Biotech LLC and supplied with the test include:

Note: The content in the kit includes supplies and reagents for 1x 10 runs.

Kit components	Description	Concentration	Volume
Positive Control	Non-infectious, recombinant viral N protein	1 ng/mL	1 mL
4x IP Lysis Buffer, pH 7.4	Lysis buffer to release and denature viral proteins	4x	7 mL
4x Viral Lysis Buffer, pH 8.5	Lysis buffer to release protein from the beads	4x	250 µL
50x Protease Inhibitor Cocktail	Protease inhibitor mix	50x	600 µL
Primary Antibody	Rabbit monoclonal Ab in PBS, pH7.4	1 mg/mL	20 µL
Magnetic Beads	Superparamagnetic beads covalently bound with recombinant protein A	25% bead slurry	66 µL
Blocking Reagent	IgG to block the magnetic beads after anti-N Ab binding	100x	20 µL

2) EQUIPMENT REQUIRED BUT NOT INCLUDED WITH THE TEST:

Required Equipment	Purposes
Magnetic Rack Separator or equivalent	Separate magnetic beads from the solution
Benchmark R2020 Roto-Mini Rotator with Tube Holders or equivalent	Suspend the magnetic beads in solution
Benchmark Scientific Microplate Orbital Shaker or equivalent	Shake the 96-well microplate
Globe Scientific 111568 Polypropylene Graduated Microcentrifuge Tube with Snap Cap, 2ml Capacity, Round Bottom, Natural or equivalent	Used for magnetic bead preclearing step
Waverly TR6-E Analog Tube Roller, Variable Speed, 6 Rollers, or equivalent	Suspend the magnetic beads in solution
Waverly C100 Mini Centrifuge, Clear Lid, or equivalent	Collect specimens to the bottom of the tube

3) INSTRUCTIONS FOR USE:

Notes: *1) All the volume is calculated based on 10 recombinant N (rN)-spiked human nasopharyngeal swab specimens (2 mL). 2) You can scale the volume up or down based on how many samples you are going to use to do the experiment. 3) We strongly suggest positive and negative controls are run together with the samples to make sure the assay is working as intended.*

Reagent Preparation

- Phosphate-buffered saline (PBS), pH 7.4, 1L
  - 8g NaCl
  - 0.2 g KCl
  - 1.44 g Na_2HPO_4

  - 0.24g KH_2PO_4
- Bead wash/binding buffer:
  1x PBS, pH 7.0, 0.1% Tween-20
- 2% Blocking Reagent:
  Add 20 µL of the Blocking Reagent to 1 mL of PBS, pH 7.4 to a final concentration of 2%.
- Cross-Linking Wash Buffer:
  0.2 M triethanolamine pH 8.2
- Cross-Linking Reagent:
  20 mM dimethyl pimelimidate dihydrochloride (DMP) in 0.2 M triethanolamine pH 8.2.
- Cross-Linking Stop Solution:
  50 mM Tris-HCl, pH 7.5
- Non-Denaturing Elution Buffer:
  0.1 M glycine, pH 2.5
- Neutralizing Buffer:
  1 M Tris-HCl, pH 8.5
- 1x Viral Lysis Buffer
  Add 250 µL of the 4x Viral Lysis Buffer to 750 µL of ddH2O. Mix thoroughly using vortexing.

Preclear Specimens

1. Prepare the Magnetic Beads as follows:
   a. Invert the tube containing the Magnetic Beads 3-5 times to mix the beads well.
   b. Immediately transfer 30 µL of the bead slurry into a 2 mL round-bottom centrifuge tube.
   c. Separate the beads from the solution using a magnetic stand; discard the supernatant.
   d. Wash the beads with 1 mL of Bead Wash/Binding Buffer.
   e. Separate the beads and buffer using a magnetic stand, discard the buffer.
   f. Repeat the above wash step *two* more times.
   g. Suspend the beads by adding 330 µL of the Bead Wash/Binding Buffer.
   h. Mix the beads thoroughly by inverting 10 times.
   i. For each sample, distribute 30 µL of the washed bead slurry into a 5 mL round-bottom centrifuge tube labelled with sample ID. Note: To ensure that each tube receives the same amount of bead slurry, gently invert the tube containing the beads 3 to 5 times each time before distributing the bead slurry.
2. Transfer 2 mL of the NP swab specimen solution to the labelled 5 mL round-bottom tube containing 30 µL of the magnetic beads from **Step 1i**.
3. Place the tubes on a laboratory rotator and let the tubes rotate continuously at room temperature (RT) for 1 h. Alternatively, the tubes can be placed on an analog or digital tube roller rotating at high speed such that the beads will not precipitate during rotation. *Note: This preclearing step is necessary particularly when you are dealing with human nasal or oral specimens*.
4. 1 h later, separate the beads and solution using a magnetic stand, discard the beads. Keep the precleared solutions.

Lyse the Precleared Specimens

5. Add the 4x IP Lysis Buffer, pH 7.4 to the precleared samples from **Step 4** at a ratio of 1 to 3 (v/v).
6. Add 50x Protease Inhibitor Cocktail to the above solution at a ratio of 1 to 49 (v/v).
7. Lyse the samples on ice for 20 min. The lysates are ready for immunoprecipitation.

Couple Antibody to the Beads

8. Prepare antibody-bead complex as follows:
    a. Invert the tube containing the Magnetic Beads 3-5 times to mix the beads well.
    b. Immediately transfer 30 µL of the bead slurry into a 2 mL round-bottom centrifuge tube.
    c. Separate the beads from the solution using a magnetic stand; discard the supernatant.
    d. Wash the beads 3 times with 1 mL of Bead Wash/Binding Buffer as described above.
    e. After the final wash, add 1 mL of Bead Wash/Binding Buffer.
    f. Add 20 µL of the primary antibody (anti-N rabbit monoclonal Ab) to the tube.
    g. Incubate the beads-antibody mix on a laboratory rotator at room temperature for 1 h.
    h. Separate the beads from the solution using a magnetic stand; discard the supernatant.
    i. Wash the beads 3 times with 1 mL of Bead Wash/Binding Buffer as described above.

9. Block antibody-bead complex as follows:
    a. Add 1 mL of the 2% Block Reagent to the bead pellet. Gently invert the tube to mix.
    b. Incubate the above mixture on a laboratory rotator at room temperature for 30 min.
    c. Separate the beads from the solution using a magnetic stand; discard the supernatant.
    d. Wash the beads 3 times with 1 mL of Bead Wash/Binding Buffer each time as described above.

10. Cross-link the blocked antibody-bead complex as follows:
    a. Add 1 mL of the Cross-Linking Wash Buffer (0.2 M triethanolamine pH 8.2) to the above antibody-bead complex.
    b. Separate the beads from the solution using a magnetic stand; discard the supernatant.
    c. Repeat the above wash step *one* more time.
    d. Resuspend the bead-antibody pellet in 1 mL of Cross-Linking Reagent (20 mM dimethyl pimelimidate dihydrochloride (DMP) in 0.2 M triethanolamine pH 8.2). *Note: The cross-linking reagent should be prepared immediately prior to use.*
    e. Incubate the above solution on a laboratory rotator at room temperature for 30 min.
    f. Separate the beads from the solution using a magnetic stand; discard the supernatant.
    g. Resuspend the beads with 1 mL of the Cross-Linking Stop Solution (50 mM Tris-HCl, pH 7.5) and incubate on a laboratory rotator at room temperature for 15 min.
    h. Separate the beads from the solution using a magnetic stand; discard the supernatant.
    i. Wash the beads 3 times with 1 mL of PBS, pH 7.4.
    j. Add 1.1 mL of PBS, pH7.4 to the tube after the last wash. Gently invert the tube to mix.

Perform Immunoprecipitation

11. Add 0.1 mL of the cross-linked antibody-bead complex from **Step 10j** to each precleared sample from **Step 7**.
12. Incubate the above mixture on a laboratory rotator at room temperature for 1 h or over-night at 4°C.
13. Briefly centrifuge the tubes and collect all the solution to the bottom using a mini centrifuge.
14. Place the specimen in a magnetic stand to separate the beads from the specimen.
15. Wash the beads 3 times with 1 mL of PBS pH 7.4.

Elute Target Protein

- Elute Target Protein in Non-Denaturing Buffer
    a. For nondenaturing elution, add 100 µL of the Non-Denaturing Elution Buffer (0.1 M glycine, pH 2.5) to the beads and incubate at RT for 5 min with constant agitation.

b. Place the specimen in a magnetic stand to separate the beads from the specimen.
c. Transfer the supernatant to a new centrifuge tube.
d. Repeat the above step *one* more time.
e. Add 10 µL of the Neutralization Buffer (1 M Tris-HCl, pH 8.5) per 100 µL of the Non-Denaturing Elution Buffer and mix well. The eluted protein solution may be stored in -20°C for future use.

- Elute Target Protein in Denaturing Buffer
  a. For denaturing elution, add 100 µL of the 1x Viral Lysis Buffer, pH 8.5 to the beads and incubate at RT for 20 min with constant agitation.
  b. Place the specimen in a magnetic stand to separate the beads from the specimen.
  c. Transfer the supernatant to a new centrifuge tube.
  d. Freeze the eluted protein solution in -20°C for future use. The denatured protein can be used for Western blotting and/or ELISA.

VALIDATION DATA

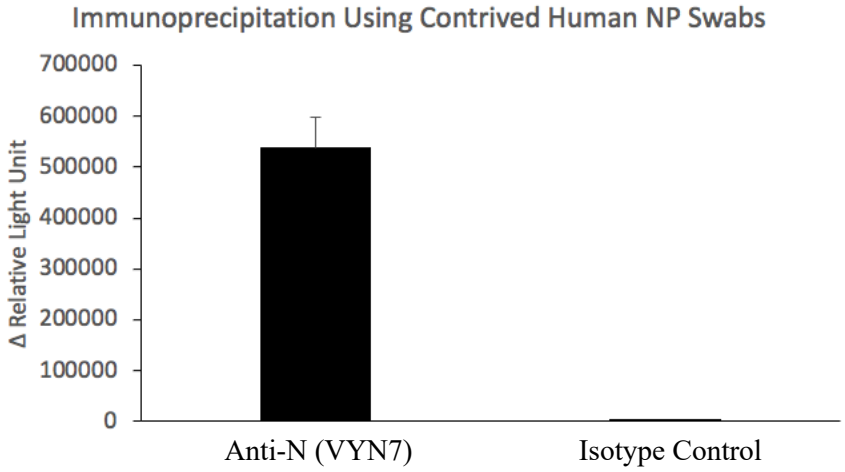

Figure 1. Immunoprecipitation (IP) using contrived human nasopharyngeal (NP) swab specimens. A recombinant N protein (rN, Cat# VPN2, 1 ng) was spiked into 2 mL of EUA RT-PCR-confirmed negative human NP swab specimen. A rabbit monoclonal anti-N antibody (Cat# VYN7, 2 µL) was used for immunoprecipitation according to the instructions in the kit. The rabbit monoclonal anti-N antibody (Cat# VYN7, 1:10,000) and an HRP-conjugated highly cross-adsorbed goat anti-rabbit IgG secondary antibody (Cat# 202, 1:20,000) were used to detect SARS-CoV-2 N protein in denatured lysates. PiQ™ ECL (Cat# 636) substrates were used to generate chemiluminescent signals. Data represent 6 independent experiments. RLU, relative light unit. ΔRLU = RLU of the well with the primary antibody - RLU of the corresponding well without the primary antibody, everything else being equal.

Table 1: Line data of the immunoprecipitation using contrived human nasopharyngeal (NP) swab specimens (n = 2).

	W/o 1°Ab	W/ 1°Ab	Δ RLU
Positive Control (rN 18 pg)	152899	231029	78130
Negative Control (1x IP Viral Lysis Buffer)	174668	156305	-18363
Rabbit Isotype Control	159005	154119	-4886
Sample No.1	802939	1248570	445631
Sample No.2	750604	1383115	632511
Contrived specimen (7.5% of the original 2 mL)	159484	195879	36395

Notes:

1. Immunoprecipitation using contrived human nasopharyngeal (NP) swab specimens. Recombinant N protein (rN, Cat# VPN2, 1 ng) was spiked into EUA RT-PCR-confirmed negative human NP swab specimens.
2. A rabbit monoclonal anti-N primary antibody (Cat# VYN7, 2 µL) was used for immunoprecipitation.
3. After IP, the beads were lysed in 1x Viral Lysis Buffer; the lysates were used for chemiluminescent ELISA with a rabbit monoclonal anti-N antibody (Cat# VYN7, 1:10,000) and an HRP-conjugated highly cross-adsorbed goat anti-rabbit IgG secondary antibody (Cat# 202, 1:20,000). PiQ™ ECL (Cat# 636) substrates was used to generate chemiluminescent signals.
4. RLU, relative light unit. Δ RLU = RLU of the well with the primary antibody - RLU of the corresponding well without the primary antibody, everything else being equal.