K4B04TE

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In Vitro Diagnostic Use

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ESPLINE® SARS-CoV-2

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8:00 – 17:00 GMT+1

M	T	W	T	F	S	S
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Read this insert carefully before performing the assay and make sure you are using the most recent version of the package insert.
The reliability of assay procedures other than those described in this package insert cannot be guaranteed.

■ NAME

ESPLINE SARS-CoV-2

■ INTENDED USE

ESPLINE SARS-CoV-2 is for *in vitro* diagnostic use with immunochromatographic assay for the detection of SARS-CoV-2 antigen directly from nasopharyngeal swab fluid and is intended for use as an aid in the diagnosis of SARS-CoV-2 infection. This product is for professional use only.

■ SUMMARY AND EXPLANATION OF THE ASSAY

The 2019 novel coronavirus infection disease (COVID-19) is caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1,2] In December 2019, the Health Commission of the City of Wuhan, Hubei Province, China, reported multiple pneumonia patients with unknown cause. On January 7th 2020, the World Health Organization (WHO) announced that the National Health Commission of China identified a new type of coronavirus, SARS-CoV-2.[3] The WHO declared a COVID-19 pandemic on March 11th 2020 due to the worldwide spread of novel coronavirus infection.[4]

The detection of viruses in the body of the patient is the most effective way of confirming SARS-CoV-2 infection. In most countries including Japan, the diagnosis of SARS-CoV-2 infection is based on the molecular detection of the SARS-CoV-2 genes. Though nucleic acid-based test can detect SARS-CoV-2 gene with high sensitivity, it is affected by the need of special equipment and the duration of reaction time. ESPLINE SARS-CoV-2 is a cassette-style assay using a simple procedure without any special instruments. SARS-CoV-2 can be detected within 30 minutes.

■ PRINCIPLE OF THE PROCEDURE

ESPLINE SARS-CoV-2 is an immunochromatographic test that uses anti-SARS-CoV-2 monoclonal antibodies. When sample is added to the cassette (sample window), SARS-CoV-2 antigens present in the sample bind to anti-SARS-CoV-2 antibodies conjugated with alkaline phosphatase (ALP) and migrate to the interpretation window area. The antigen-antibody complexes are captured by the anti-SARS-CoV-2 antibodies immobilized on the SARS-CoV-2 test line. The ALP enzyme reacts with the substrate and forms a blue colored SARS-CoV-2 test line (T) in the interpretation window. Excess ALP labeled antibodies continue to migrate on the membrane and a blue colored reference line (r) appears.

■ MATERIALS PROVIDED

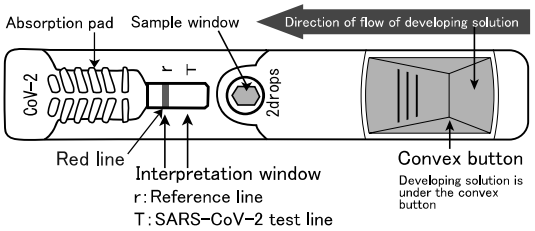


Fig.1 Reaction Cassette

ESPLINE SARS-CoV-2 REF 231906

Kits Components / Package Specification	Reagents		
	CoV-2	I	TIP
100 Tests	10 × 10 Tests/Box	200µL/Tube 20×5 Tubes/Bag	10 × 10 Tips/Bag

CoV-2 SARS-CoV-2 Reaction Cassette:
Anti-SARS-CoV-2 monoclonal antibodies (mouse)
Alkaline phosphatase (ALP)-labeled anti-SARS-CoV-2 monoclonal antibodies (mouse)
5-bromo-4-chloro-3-indolyl phosphate disodium salt
Sodium azide 0.05% (w/v)

I Sample Extraction Solution (Squeeze Tube):
Surfactant and Bovine Serum Albumin,
Sodium azide 0.095% (w/v)

TIP Applicator Tip

■ MATERIALS REQUIRED BUT NOT PROVIDED

The following swabs were recommended for use in the ESPLINE SARS-CoV-2.

●Sterile swabs (CE marking)
Nasopharyngeal swab fluid:

- Dry Swabs 170KS01 Regular Rayon Swab w/ Aluminium Applicator (Catalog No. 170KS01. Copan Italia S.p.A, Italy)
- Puritan 5.5" Sterile Mini-tip Rayon Swab w/ Aluminum Handle (Catalog No. 25-800 R 50. Puritan Medical Products, US)
- Peel Pouch Dry swab ENT (Catalog No.MW151. Medical Wire & Equipment, UK)

■ WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use only

1. SAFETY PRECAUTIONS

Please refer to the safety data sheet (SDS) and product labeling for information on potentially hazardous components. The most recent SDS version is available on the website www.fujirebio-europe.com.

H412: Harmful to aquatic life with long lasting effects.

2. PRECAUTIONS FOR HANDLING

1) All specimens should be handled as infectious materials. Disposable gloves, mask, goggles and proper protective clothing should be worn.
2) If infection with a novel SARS-CoV is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent SARS-CoV and sent to local health departments for testing.
3) Do not use a Reaction Cassette on which the red line is not next to the letter “r” or on which the red line is not clear (or not visible). Refer to Fig.1.

4) The developing solution in Reaction Cassette and Sample Extraction Solution contain sodium azide as a preservative (0.05%, 0.095%). Avoid direct contact with the skin or eyes. In the event of accidental contact of the reagent with eyes or mouth, rinse thoroughly with water, and seek medical treatment if necessary.
5) Do not drink, eat, or smoke in areas where specimens are being handled or testing is being performed.
6) Do not touch or damage the sample window and the interpretation window of the Reaction Cassette. The aluminum pouch contains a Reaction Cassette, which has a convex button, interpretation window, and sample window. Handle the pouch gently and carefully. Do not press the pouch with the cassette inside. Do not place anything on top of the pouch.
7) Keep the Reaction Cassette away from sources of ignition.
8) Remove the Sample Extraction Solution (Squeeze Tube) from the plastic zipper bag immediately before testing. For unused squeeze tubes, be sure to close the zipper at the opening of the plastic zipper bag and store under the specified conditions (storage temperature 1-30 °C).

3. PRECAUTIONS FOR USE

1) Follow the instructions described in this package insert.
2) Do not use the kit beyond the expiration date that appears on the kit.
3) Do not reuse any of kit components.
4) Invalid results may be obtained when a specimen is collected with another swab than the one recommended. Do not use the swab if it is visibly damaged.

4. PRECAUTIONS FOR WASTE

1) The reagents contain sodium azide as a preservative as previously described. Sodium azide has been reported to form explosive lead and copper azides in laboratory plumbing. To prevent azide buildup, flush with large volumes of water if solution containing azide are disposed of in the sink.
Follow any applicable regulations for disposal.
2) Handle any medical waste in compliance with waste-related regulations.

■ STORAGE INSTRUCTIONS

Store at 1-30 °C. DO NOT FREEZE
Keep away from direct sunlight.
When stored and handled properly, reagents are stable until the expiration date. Refer to the expiration date shown on the immediate container label.

■ SPECIMEN COLLECTION AND PREPARATION

Inadequate or inappropriate specimen collection is likely to yield false negative test result. Collect nasopharyngeal swab fluid specimen adequately and appropriately.
Refer to medical books.

1. Specimen collection

Nasopharyngeal swab fluid specimen
Insert the sterile swab deeply into the nasopharyngeal cavity from the nostril to reach adenoids and rotate the swab several times to rub the nasopharyngeal turbinates and collect epidermis from the mucosa. Samples must be prepared immediately after specimen collection.

2. Sample preparation

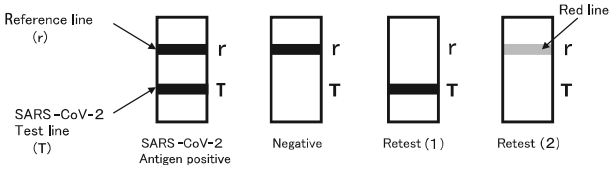
Allow the Sample Extraction Solution to reach a temperature range of 20 to 37 °C prior to use and peel off the blue top seal. Samples must be prepared immediately after specimen collection.
1) Place the swab into the Sample Extraction Solution (Squeeze Tube) and squeeze the swab from outside of the Squeeze Tube approximately 10 times. Finally, squeeze the swab head to extract the sample.
2) Insert the Applicator Tip securely onto the top of the tube containing the Sample Extraction Solution.
3) Let sample stand for about 5 minutes.

■ ASSAY PROCEDURE

Allow the Reaction Cassette to reach a temperature range of 20 to 37 °C prior to testing. Do not open the aluminum pouch until ready to perform the assay.
1. Take the Reaction Cassette out of the aluminum pouch.
Note: Hold the edge of the aluminum pouch when opening. Be careful to avoid pressing the convex button, which starts the reaction.
2. Hold Squeeze Tube vertically (approximately 10 mm above the Reaction Cassette) and apply two drops of Squeeze Tube of the sample onto the sample window of the Reaction Cassette.
3. Press the convex button of the Reaction Cassette down immediately after applying the sample to start the reaction.
4. Leave the cassette horizontally for 30 minutes.
5. Interpret the result at 30 minutes. (Refer to ■ **RESULTS**)
Note: If the reference line and the test line appear before 30 minutes, the sample must be considered "positive". A sample that is "negative" after the 30-minute reaction time and then turns "positive" after 30 minutes, must be considered "negative".

■ RESULTS

1. Positive
SARS-CoV-2 Positive: a blue reference line is observed at r position and a blue test line appears at the T position.
2. Negative
A blue reference line is observed at r position but no blue test line appears at the T position.
3. Invalid
Retest (1): There is no blue reference line at 30 minutes
Retest (2): There is still a red reference line at 30 minutes
Review the test procedure and repeat the test using a new Reaction Cassette.
If the problem persists, contact your local distributor.



Note: A comprehensive diagnosis has to be made by taking into account epidemiological information, clinical signs and symptoms.

■ LIMITATIONS OF THE PROCEDURE

1. Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely during peak activity when prevalence of disease is high. False positive test results are more likely during periods of low SARS-CoV-2 activity when prevalence is moderate to low.
2. Monoclonal antibodies may fail to detect, or detect with less sensitivity, SARS-CoV-2 that have undergone minor amino acid changes in the target epitope region.
3. ESPLINE SARS-CoV-2 is designed for the qualitative detection of SARS-CoV-2 antigens from nasopharyngeal swab fluid. The results obtained with ESPLINE SARS-CoV-2 should be used in conjunction with clinical signs, symptoms, and other test results to make an accurate diagnosis.
4. A negative test result may occur if the level of antigen in a sample is below the detection limit of the test. Test results must be evaluated in conjunction with other clinical data and prevalence information of COVID-19 available to the physician.
5. Negative results do not preclude SARS-CoV-2 and other virus infection.
6. ESPLINE SARS-CoV-2 uses antibodies to nucleocapsid proteins of SARS-CoV-2. The results from ESPLINE SARS-CoV-2 can differ from those from virus isolation and the PCR method. The virus isolation method confirms the presence of virus by cultivating the virus in the sample, and the PCR method confirms the presence of virus by amplifying nucleic acid of the virus.

■ PERFORMANCE CHARACTERISTICS

☆ 1. Clinical data

To evaluate the accuracy of the ESPLINE SARS-CoV-2, a correlation with RT-PCR using nasopharyngeal samples was carried out in two external centers.
1) In the first center (Japan)[5], 162 nasopharyngeal samples were evaluated.
The overall concordance was 92.6% (150/162; 95% CI: 87.5-95.7%) with a specificity of 100.0% (100/100; 95% CI: 96.3-100.0%) and an overall sensitivity of 80.6% (50/62; 95% CI: 69.1-88.6%). The mean Ct value was 24.9 ± 5.45. Samples with a Ct value ≤27 had a sensitivity of 97.6% (40/41; 95% CI: 87.4-99.6%).

		RT-PCR Ct-value range			
		≤25	≤27	≤30	≤40
ESPLINE SARS-CoV-2	Positive	32	40	48	50
	Negative	0	1	2	12
	Total	32	41	50	62
Sensitivity		100.0%	97.6%	96.0%	80.6%

When comparison was based on the positivity rate of RNA copies, the positivity rate of RNA copies higher than 6400 was 100.0% (39/39; 95% CI: 91.0-100.0%), for RNA copies higher than 1600 was 97.9% (46/47; 95% CI: 88.9-99.6%), for RNA copies higher than 400 was 96.0% (48/50; 95% CI: 86.5-98.9%), and for RNA copies higher than 100 was 87.5% (49/56; 95% CI: 76.4-93.8%).

	RNA copies				Total
	>6400	>1600	>400	>100	
Number of positive specimens (N=62)	39	47	50	56	62
ESPLINE SARS-CoV-2 Positive	39	46	48	49	50
ESPLINE SARS-CoV-2 Negative	0	1	2	7	12
Concordance rate	100.0%	97.9%	96.0%	87.5%	80.6%

2) In the second center (France), 245 nasopharyngeal samples were evaluated.
Of the 245 samples tested, 119 (48.6%) were negative and 126 (51.4%) were positive. For 194 patients, the clinical status was known and is shown in table below.

RT-PCR result	Negative	Positive	Total
Asymptomatic patient	30 (15.5%)	5 (2.6%)	35 (18.0%)
Symptomatic from 0 to 1 day	5 (2.6%)	18 (9.3%)	23 (11.9%)
Symptomatic for 2 to 4 days	33 (17.0%)	73 (37.6%)	106 (54.6%)
Symptomatic for 5 to 7 days	14 (7.2%)	16 (8.2%)	30 (15.5%)
No information	37	14	51
Total	119 (48.6%)	126 (51.4%)	245

The overall concordance was 82.9% (203/245; 95% CI: 77.6-87.1%) with a specificity of 100.0% (119/119; 95% CI: 96.9-100.0%) and an overall sensitivity of 66.7% (84/126; 95% CI: 58.1-74.3%). The mean Ct value was 24.3 ± 7.21. Samples with a Ct value ≤27 had a sensitivity of 87.8% (79/90; 95% CI: 79.4-93.0%).

		RT-PCR Ct-value range		
		≤27	≤33	All
ESPLINE SARS-CoV-2	Positive	79	82	84
	Negative	11	24	42
	Total	90	106	126
Sensitivity		87.8%	77.4%	66.7%

A total of 188 positive samples and 219 negative samples were assessed in both centers. Overall, the specificity in each of the studies was 100.0% (219 samples) and the sensitivity in each of the studies was ≥87.8% respectively for samples with Ct ≤27 (131 samples).

☆**2. Cross reactivity**

The ESPLINE SARS-CoV-2 assay was evaluated for cross-reactivity using potentially cross-reactive organisms spiked into a negative sample pool at concentrations greater than or equal to 10^5 pfu/mL or 10^6 CFU/mL (see table below). No cross-reactivity was observed for the tested cross-reactants.

Potential Cross-Reactant	**Required Test Concentration***
Adenovirus 21	≥10^5 pfu/mL
Human Metapneumovirus (HMPV)	≥10^5 pfu/mL
Parainfluenza Virus Type 1	≥10^5 pfu/mL
Human Parainfluenza Virus 2	≥10^5 pfu/mL
Human Parainfluenza Virus 3	≥10^5 pfu/mL
Human Parainfluenza Virus 4B	≥10^5 pfu/mL
Influenza A Virus (H3N2)	≥10^5 pfu/mL
Influenza A Virus (H1N1)	≥10^5 pfu/mL
Influenza B Virus	≥10^5 pfu/mL
Enterovirus D	≥10^5 pfu/mL
Respiratory Syncytial Virus Type A (RSV-A)	≥10^5 pfu/mL
Rhinovirus 34	≥10^5 pfu/mL
Hemophilus Influenza Type B	≥10^6 CFU/mL
Streptococcus Pneumoniae	≥10^6 CFU/mL
Streptococcus Pyogenes	≥10^6 CFU/mL
Candida Albicans	≥10^6 CFU/mL
Pooled Human Nasal Wash	50%
Bordetella Pertussis	≥10^6 CFU/mL
Mycoplasma Pneumoniae	≥10^6 CFU/mL
Chlamydophila Pneumoniae	≥10^6 CFU/mL
Legionella Pneumophila	≥10^6 CFU/mL
Human Coronavirus 229E	≥10^5 pfu/mL
Human Coronavirus OC43	≥10^5 pfu/mL
Human Coronavirus NL63	≥10^5 pfu/mL
MERS-CoV	≥10^5 pfu/mL
Pneumocystis Jiroveci	≥10^6 CFU/mL
Staphylococcus Aureus	≥10^6 CFU/mL
Staphylococcus Epidermis	≥10^6 CFU/mL

*Due to lot availability, the final test concentration for Human Coronavirus NL63 was lower than the required test concentration of ≥10^5 pfu/mL.

3. Sensitivity
Three in-house control samples were tested in 3 replicates on 3 lots. All 3 samples gave positive SARS-CoV-2 results.

4. Accuracy
Four in-house control samples (1 negative, 3 positives) were tested in 3 replicates on 3 lots. The negative in-house control sample gave a negative result and all 3 positive in-house control samples gave positive results.

5. Reproducibility
Four in-house control samples (1 negative, 3 positives) were tested 3 times repeatedly. All samples gave each time the same result.

6. Detection Limit
25 pg/mL.

7. Materials used for calibration
In-house standard products.

☆■ INTERFERING SUBSTANCES

ESPLINE SARS-CoV-2 demonstrated interference for each compound shown below, in a study consistent with the guidelines in the CLSI document EP07-A3.[6)]

Endogenous Interferences

Whole blood	5%
Hemoglobin	≥450 mg/dL

■ BIBLIOGRAPHY

1. Wu F, *et al*. A new coronavirus associated with human respiratory disease in China. Nature, 579: 265-269, 2020.
2. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol, 5: 536-544, 2020.
3. WHO website "Rolling updates on coronavirus disease(COVID-19)" (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/events-as-they-happen) Medical Research and Development.
4. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020. (https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020)

☆5. Takeda Y, *et al*. SARS-CoV-2 qRT-PCR Ct value distribution in Japan and possible utility of rapid antigen testing kit. (https://doi.org/10.1101/2020.06.16.20131243)

☆6. Clinical and Laboratory Standards Institute. Interference Testing in Clinical Chemistry. Third Edition. CLSI guideline EP07-A3.

■ GLOSSARY OF SYMBOLS

CE	CE Marking (European directive 98/79/EC on *in vitro* diagnostic medical devices)		
EC REP	Authorised Representative in the European Community		
IVD	*In Vitro* Diagnostic Medical Device	LOT	Batch Code
Σ n	Contains Sufficient for<n>Tests		Consult Instructions for Use
REF	Catalogue Number		Use by
SN	Serial Number		Do not reuse
	keep away from sunlight		Temperature Limitation
	Manufacturer		Fragile,handle with care
	Caution		Open here
CONT	Contents of the Kit	CoV-2	SARS-CoV-2 Reaction Cassette
I	Sample Extraction Solution (Squeeze Tube)	TIP	Applicator Tip

FUJIREBIO INC.

CE