



K4B03TE

In Vitro Diagnostic Use

☆ KEY-CODE: FRI70764

☆ Nov. 2020 (ver. 2)

☆ : Note changes

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ESPLINE® SARS-CoV-2



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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⁾

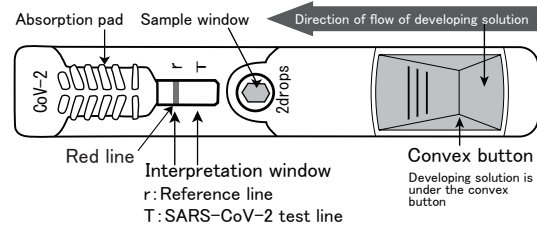
The detection of viruses in the body of the patient is the most effective way of confirming SARS-CoV-2 infection. In most countries including Japan, the diagnosis of SARS-CoV-2 infection is based on the molecular detection of the SARS-CoV-2 genes. Though nucleic acid-based test can detect SARS-CoV-2 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	Reagents		TIP
	CoV-2	I	
Package Specification	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.

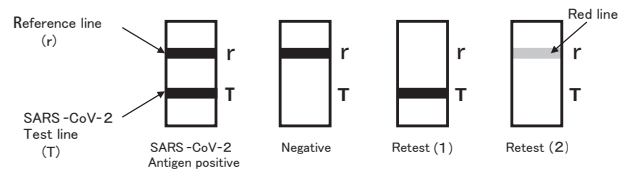
3. Invalid

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 nucleoproteins of SARS-CoV-2 antigen. 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

Correlation with RT-PCR using first visit nasopharyngeal samples ⁵⁾

To evaluate the accuracy of the device, the correlation between ESPLINE SARS-CoV-2 and RT-PCR method was evaluated using 162 first visit nasopharyngeal samples. The concordance rate between assays was 100.0% (100/100) for negative samples and above 80.0% (50/62) for positive samples with an overall concordance of 92.6% (150/162). For the indices of accuracy, the sensitivity was 80.6% (95% CI 69.1-88.6%), the specificity was 100.0% (95% CI 96.3-100.0%), with positive predictive value (PPV) of 100.0% (50/50, 95% CI 92.9-100.0%), negative predictive value (NPV) of 89.3% (100/112, 95% CI 82.2-93.8%), and overall percentage agreement of 92.6% (150/162, 95% CI 87.5-95.7%).



When comparison was based on the positivity rate according to Ct value: The positivity rate of specimens with Ct values up to 25 was 100% (32/32, 95% CI 89.3-100.0%), for Ct values up to 30 was 96.0% (48/50, 95% CI 86.5-98.9%) and for Ct values up to 40 was 80.6% (50/62, 95% CI 69.1-88.6%).

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

When comparison was based on the positivity rate of RNA copies: The positivity rate of RNA copies more than 6400 was 100.0% (39/39, 95% CI 91.0-100.0%), for RNA copies more than 1600 was 97.9% (46/47, 95% CI 88.9-99.6%), for RNA copies more than 400 was 96.0% (48/50, 95% CI 86.5-98.9%) and for RNA copies more than 100 was 89.1% (49/55, 95% CI 78.2-94.9%)

All the 100 RT-PCR negative samples were negative on ESPLINE SARS-CoV-2.

Noteworthy, for these samples, the same sample solution was used for the two assays (The swab has been immersed in the virus transport solution then used on ESPLINE SARS-CoV-2).

	RNA copies				Total
	>6400	>1600	>400	>100	
Number of positive specimens (N=62)	39	47	50	55	62
ESPLINE SARS-CoV-2 Positive	39	46	48	49	50
ESPLINE SARS-CoV-2 Negative	0	1	2	6	12
Concordance rate	100.0%	97.9%	96.0%	89.1%	80.6%

2. Cross reactivity

This reagent does not show any cross reactivity with inactivated Influenza viruses (Influenza virus H1N1, Influenza virus H3N2, Influenza virus B) and in-house recombinant human coronavirus antigens up to 200 ng/mL (MERS-CoV, HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1). The reactivity of the reagent is confirmed with in-house recombinant human coronavirus antigen SARS-CoV. However, we have not examined the reactivity with the native virus antigens.

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

Data shows that up to 450 mg/dL of hemolyzed hemoglobin and up to 2% of whole blood do not interfere with the performance of ESPLINE SARS-CoV-2.

■ 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. Omi K, *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>)

■ GLOSSARY OF SYMBOLS

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

ESPLINE is a registered trademark of Fujirebio Inc. in Japan and in other countries.