CO-DIAGNOSTICS Logix Smart ABC Test Kit User Manual

Contents

CO-DIAGNOSTICS Logix Smart ABC Test Kit

INTENDED USE

The Logix Smart ABC (Influenza A/B, SARS-CoV-2) Test kit is a research use only multiplex test, based on real-time PCR (qPCR) technology, for the simultaneous qualitative detection of the Influenza A (INFA), Influenza B (INFB), and SARS-CoV-2 (COVID-19) specific RNA.

For research use only (RUO). Not for use in diagnostics procedures.

KIT COMPONENTS

Lid Color	Component	Symbol	Catalog Number	Description	Amount
Brown	Logix Smart™ ABC Master Mix	MM	ABC-MM- 001	Proprietary blend of CoPrimers™ and PCR reagents	1x1000µL (100 reactions)
Red	Logix Smart™ ABC Positive Control	PC	ABC-PC- 001	Proprietary blend of target templates	1x1000µL (100 reactions)
Clear	Nuclease-Free Water	NTC	GEN-NF-001	DNase/RNase-free water	1x1000µL (100 reactions)

Kit Catalog Number is ABC-K- 001. Contact Sales at 801-438-1036 ext. 02 or at www.codiagnostics.com/contact/toorder.

LOGIX SMART™ ABC STORAGE, HANDLING, & DISPOSAL

- The Logix Smart™ ABC kit is shipped on dry ice. The components of the kit should arrive frozen. If one or more of the components are not frozen upon receipt, or are compromised during shipment, contact your distributor for assistance.
- Upon receipt of kit, laboratory should follow internal procedures for quality control.

- All components should be stored below -20°C upon arrival to prevent degradation of reagents.
- Repeated thawing and freezing of components (more than four times) should be avoided, specifically the master mix, as this might affect the performance of the assay. The reagents should be frozen in multiple aliquots if they are to be used intermittently.
- Co-Diagnostics recommends storage between +2°C and +8°C should not exceed a period of 4 hours.
- If you work in an area prone to power outages, it is recommended to have a back-up generator for your freezer as well as a temperature data log system to ensure that the Logix Smart™ ABC test kit remains frozen at -20°C.
- Protect Master Mix from light.
- Expired products should not be used, as the integrity of the components cannot be guaranteed.
- The product is not a biological waste. See Safety Data Sheets (SDS) for hazard classification. Disposal should be in accordance with applicable regional, national, and local laws and regulations.

WARNINGS AND PRECAUTIONS

Read this Instructions for Use carefully before using the product. Before first use check the components for:

- Integrity
- Frozenness upon arrival

Users should pay attention to the following

- Use of this product should be limited to personnel instructed and trained in the techniques of real-time PCR.
- Samples should always be treated as infectious and/or biohazardous. Use standard precautions.
- Wear protective gloves, lab coat, and eye protection when handling samples. Always wear gloves when handling kit components.
- Always use DNase/RNase-free disposable pipette tips with filters.
- Use segregated working areas for sample preparation, reaction setup, and amplification/detection activities. The workflow in the laboratory should proceed in a unidirectional workflow. To prevent contamination, change PPE between areas.
- Store and extract positive materials (specimen, controls, and amplicons) separately from other reagents. Dedicate supplies and equipment to separate working areas and do not move them from one area to another.
- Consult appropriate Safety data Sheets (SDS) for safety. The SDS for the Logix Smart™ ABC test kit is provided with the shipment. If not provided with shipment the SDS can be retrieved from Co-Diagnostics website at the link: **http://codiagnostics.com/resources/safety-data-sheets/**
- Do not open the reaction tubes/plates post amplification.
- Do not autoclave reaction tubes/plates after the PCR, since this will not degrade the amplified nucleic acid and will pose a risk to the laboratory area to contamination.
- Do not use components of the kit that have passed expiration date.
- Discard sample and assay waste according to your local safety regulations.

PRODUCT DESCRIPTION

The Logix Smart™ ABC kit is a research use only multiplex test, based on real-time polymerase chain reaction

technology. It tests for the presence or absence of ribonucleic acid (RNA) of the Influenza A, Influenza B, and SARS-CoV-2 viruses. The Logix Smart™ ABC test includes an internal control to identify possible qPCR inhibition, confirm the integrity of the reagents, and verify the quality of sample extraction. The Logix Smart™ ABC test also includes a positive control which includes three synthetic RNA molecules carrying sequences that are homologous to Influenza A (INFA), Influenza B (INFB), and SARS-CoV-2 (COVID-19) viruses and are targeted by this multiplex assay. Positive controls represent a source of cross-contamination. Precautions should be taken to prevent and minimize the risk.

CoPrimers™ included in the Logix Smart™ ABC test are:

- CoPrimers™ that are targeting INFA are labelled with the Quasar® 670 fluorophore
- CoPrimers™ that are targeting INFB are labelled with the CAL Fluor® Orange 560 fluorophore
- CoPrimers™ that are targeting COVID-19 are labelled with the FAM™ fluorophore
- CoPrimers™ that are targeting the Internal Positive Control (IPC) DNA are labelled with CAL Fluor® Red 610 fluorophore

MATERIALS AND DEVICES (REQUIRED BUT NOT PROVIDED)

- Appropriate 4-channel real-time PCR instrument, compatible with the fluorophores used in this test.
- Appropriate nucleic acid extraction system or kit
- Vortex mixer
- Centrifuge with a rotor for 2 mL reaction tubes
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)
- Ice
- Biosafety cabinet, ideally BSL-2 facility

All instruments should be properly installed, calibrated, and maintained according to the manufacturer’s instructions and recommendations. Do not use instruments with outdated calibration.

PROCEDURE

Sample Preparation

The quality of the extraction of the RNA from the samples is essential for the performance of Logix Smart™ ABC. The extraction protocol to be followed should be performed following manufacturer’s instructions or an internally validated protocol. The suitability of the nucleic acid extraction procedure for use with Logix Smart™ ABC must be validated by the user.If your sample preparation system is using washing buffers containing ethanol, make sure to eliminate any traces of ethanol prior to elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR. The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.

Logix Smart™ ABC Reagent Setup

- When preparing reagents, clean all working surfaces with a fresh 10% bleach solution followed by molecular grade alcohol, or another equivalent method of cleaning that disinfects and degrades nucleic acids.
- All Logix Smart™ ABC Master Mix, Positive Control (PC), no template control nuclease-free water (NTC), and sample tubes should be vortexed for 3 seconds, and briefly spun down before using to ensure properly mixed

reagents, and to remove any condensation or residue from the lids.

- Thaw all reagents and samples on ice, or on a cold block, before starting setup.

Reaction Setup

- Every reaction setup should include enough reaction wells for the number of samples and at least one positive and one NTC (# samples + 2 = total reaction wells needed). Example: 5 samples to test + 1 PC well + 1 NTC well = 7 total reaction wells.
- All pipetting should be done on ice, if possible. Pipetting of PC and sample elution is recommended to be done in a separate area, if possible, or at a separate time, then the Master Mix and NTC.
- Change pipette tips in-between sample elution and change pipette tips after pipetting each component. Pipet PC last if possible, to avoid contamination events.
- Pipet 10 µL of Master Mix into each well being used in an appropriate optical plate or optical reaction tube (example: CoDx Box real-time PCR instrument uses 48-well reaction tubes).
- Pipet 10 µL of sample (elution from nucleic acid extraction) or 10 µL of a control (NTC and Positive Control) to the appropriate well(s). At least one positive and one NTC control must be included in each run.
- Seal the reaction plate with an optical adhesive film or the reaction tubes with appropriate lids.
- Place plate or tubes into real-time PCR instrument in the correct orientation and start run.

PCR Instrument Setup

For basic information regarding setup and programming of the different real-time PCR instruments, please refer to the user manual of the respective instrument. For programming instructions questions regarding the use of other real-time PCR instruments please contact the Laboratory 801-438-1036 ext.04 or at **www.codiagnostics.com/contact/**.

If using Co-Diagnostics Inc. CoDx Box, contact the Laboratory 801-438-1036 ext. 04 or at **www.codiagnostics.com/contact/** for the template file for download. The template file comes pre-programmed with the PCR instrument setup described in this section. When not using a template, or using another device, use the settings outlined below to program the PCR instrument.

In order to achieve optimal performance from the test, it is important to make sure that the instrument is compatible with the conditions outlined below.

Define the following settings

Reaction Volume	20 µL
Ramp Rate	Default
Passive Reference	None

Program PCR instrument with the cycling conditions below

	Stage	Cycles	Temperature	Time
Reverse Transcription	Activation	1	45°C	15 minutes
Initial Denaturation	Hold	1	95°C	2 minutes
Amplification	Cycling	45	95°C	3 seconds
			55°C	32 seconds

Ensure that PCR instrument being used is compatible with fluorophores below. Some devices may not have options for the quencher. If needing help or have questions, contact Co-Diagnostics Inc. technical support at 801-438-1036 ext. 04 or at: [www.codiagnostics.com/contact/.](www.codiagnostics.com/contact/)

Define the fluorescence detectors (dyes):

Target	Detector Name	Reporter	Quencher
INFA specific RNA	INFA	Quasar® 670	BHQ® - 2
INFB specific RNA	INFB	CAL Flour® Orange 560	BHQ® - 1
COVID-19 specific RNA	COVID-19	FAM™	BHQ® - 1
RNaseP specific DNA (IPC)	RNaseP	CAL Flour® Red 610	BHQ® - 2

- When the run is finished, ensure that the run file is saved

DATA ANALYSIS

For basic information regarding data analysis on specific real-time PCR instruments please refer to the user manual of the respective instrument.

Validity of Test Runs

- Valid Test Run
- Check to see that both the positive and no template control passed

The following control conditions must be met:

Control Type	Control Name	Purpose of Control	INFA	INFB	COVID-19	Internal Control (RNaseP)
ABC Positive Control	INFA (Quasar®670)	Verifies the performance of the master mix	+	+	+	+
	INFB (CF®560)					
	COVID-19 (FAM™)					
	RNaseP (CF®610)					
No Template Control	Master Mix + Water	Verifies the reagents are free of contamination	-	-	-	-

If controls pass, interpret the sample results

Invalid Test Run If any of the controls fail, the run is invalid. Document the run and initiate troubleshooting.

Interpretation of Results

Once the controls have passed, the unknown samples can be interpreted based on three possible outcomes:

- Positive
- Negative
- Invalid

A Positive result will show an amplification curve or cycle threshold value for INFA, INFB, or COVID-19. The cut off value should be determined by in house validation testing. However, internal studies have shown rare primer-dimer formation or other non-specific amplification at 45 cycles. This fact can be attributed to the nature of the CoPrimers™ (Satterfield, 2014) (Poritz & Ririe, 2014). The amplification of the RNaseP (IPC) shows that the extraction was successful.
A Negative result will show no amplification for INFA, INFB, or COVID-19; The absence of a curve for ABC indicates a negative result ONLY when the RNaseP (IPC) marker is positive.
An Inconclusive result will result if any of the controls fail. See troubleshooting.

The interpretation of results can be translated to the following table

	SARS-CoV-2	Influenza A	Influenza B	Patient Internal Positive Control (RNaseP)	Logix Smart™ Positive Control	No Template Control (NTC) Logix Smart™ Master Mix + Nuclease-Free Water	Result
Instrument Reading	+	+	+	+	+	-	ABC +
	-	-	-	+	+	-	ABC -
	+	-	-	+	+	-	COVID-19 + INF A - INF B -
	-	+	-	+	+	-	COVID-19 - INF A + INF B -
	-	-	+	+	+	-	COVID-19 - INF A - INF B +
	+	+	-	+	+	-	COVID-19 + INF A + INF B -
	-	+	+	+	+	-	COVID-19 - INF A + INF B +
	+	-	+	+	+	-	COVID-19 + INF A - INF B +
	Any Result (+/-)			-	+	-	Inconclusive: See Troubleshooting
				+	-	-	
				+	+	+	

Anything before 40 cycles is considered a positive reading (+). Anything after 40 cycles is considered a negative reading (-). When possible, always check that the medical history and/or symptoms match with the result prior to treatment.

TROUBLESHOOTING

Co-Diagnostics Inc. values customer feedback and we would like to be informed of any issues with the Logix Smart™ ABC kit, even if the recommended steps for troubleshooting solve the issue. To give feedback please fill out the Customer Feedback Form by visiting **www.codiagnostics.com/contact/feedback/**

Stability

Real-time and accelerated shelf-life and in-use stability studies are currently under testing. Presently, the expiration date of this product has been established as 12 months. It is not recommended to use expired kit reagents, doing so may lead to inaccurate results.Always use the most recent version of this document for updates as more stability information will be added when studies are completed.

User Errors

Good Laboratory Practices for Molecular Biology Diagnostics (Viana & Wallis, 2011) are necessary for the use of this product. This product is not intended to be used by untrained personnel.
It is essential for the user to have some molecular biology experience and be familiar with proper pipetting technique to prevent errors, such as splashes, crossover contamination, and errors on volume selection. Pipette tips must be replaced after every pipetting. Gloves must be replaced often. Equipment, such as pipettes and real-time PCR instruments, should be calibrated when applicable. 90 minutes of online training for Good Laboratory Practices for Molecular Genetics Testing (Centers for Disease Control and Prevention, 2017) is available at the CDC website at the following link **https://www.cdc.gov/labtraining/training-courses/good-lab-practices-molecular-genetics-testing.html**

Invalid Results/Inconclusive Results

Logix Smart™ ABC Positive Control not amplifying

No amplification from the PC could be the result of one or multiple factors, such as:

- Pipetting errors (pipetting control into the wrong well, missing a well, pipetting inadequate amount of reagent),
- Incorrect placement of plates or tubes into the real-time PCR instrument,
- Logix Smart™ ABC Master Mix or Logix Smart™ ABC Positive Control degradation (result of reagents being at temperatures above -20°C for an extended period),
- Use of expired reagents,
- or the wrong reagents being used.

Without further evidence, it is best to disregard the results from the samples and re-test by re-amplification. If the positive control fails again, then an investigation should be conducted to identify possible causes for error, and the test must be reprocessed from extraction or not, depending on the investigation results and risks identified in the process. If failure of the positive control happens a third time after re-extraction and re-amplification, open a new Logix Smart™ ABC Positive Control or Master Mix, and retest. If still failing, please contact Co-Diagnostics Inc. technical support by calling 801-438-1036 ext. 04 visiting **www.codiagnostics.com/contact/**.

The Internal Positive Control (IPC) RNaseP not amplifying in samples

No amplification from the RNaseP channel could be the result of one or multiple factors, such as:

- Not enough nuclear material in the patient sample,

- PCR inhibitors such as ethanol and heparin,
- the extraction was performed incorrectly,
- or the extraction kit used is not compatible or has a step that eliminates RNaseP DNA.
- **Note:** Positive amplification in the COVID-19 channel indicates a positive result despite the lack of concurrent amplification in the IPC channel. The IPC amplification is dependent on the presence of human genomic DNA (gDNA) in the extraction sample, the amount of which is governed by the type of the patient sample and the extraction procedure used. Samples obtained from culture or sterile/pure sites (e.g. CSF, urine, cell lysates, etc.) may not contain the human RNaseP gene.

The results should be interpreted as invalid and re-testing by re-amplification should be performed. If the IPC fails again, then samples should be re-extracted and re-amplified. If it fails a third time an investigation should be conducted to identify possible causes for the error. If the cause for the error is clear, the test can either be singled out as invalid due to either PCR inhibitors being present or not enough nuclear material being present. If the cause for an error is unclear, contact Co-Diagnostics Inc. technical support by calling 801-438-1036 ext. 04 or contact us at **www.codiagnostics.com/contact/**.

No Template Control showing amplification

Amplification of ABC in a No Template Control indicates contamination in one or more of the reagents, incorrect placement of plate or tube into the real-time PCR instrument, or pipetting errors. None of the results can be trusted and re-testing by re-amplification should be performed. If the NTC fails again, then an investigation should be conducted to identify possible causes for error, and the test must be reprocessed from extraction or not, depending on the investigation results and risks identified in the process. If failure of the NTC, after re-extraction and re-amplification, happens a third time, open a new nuclease-free water and retest. If still failing, please contact Co-Diagnostics Inc. technical support by calling 801-438-1036 ext. 04 or by visiting **www.codiagnostics.com/contact/**.

LIMITATIONS

- This product is intended for research use only. Not intended for use in clinical diagnostics for its performance for diagnostic applications has not be established.
- Strict compliance with this document is required for optimal results. Please, always use the most recent version of this document. This can be downloaded for free at: **www.codiagnostics.com/resources/instructions-for-use**/ or by visiting **www.codiagnostics.com/contact/**.
- Use of this product is to be limited to trained and instructed personnel in real-time PCR techniques.
- Good laboratory practices are essential for the proper performance of this assay. It is also recommended that upon receipt of reagents a test run be performed to check the integrity, and performance of the reagents prior to testing on samples.
- Appropriate collection, transport, storage, and processing procedures of samples are required for optimal results.
- Do not use the Logix Smart™ ABC kit components directly on the specimens collected. Perform an appropriate nucleic acid extraction prior to using this assay.
- The presence of PCR inhibitors may cause false negatives or invalid results.
- Potential mutations of the target regions of the INFA, INFB, and COVID-19 genome covered by this test kit may result in failure to detect the presence of the pathogens.

QUALITY CONTROL

In accordance with the Co-Diagnostics Inc.'s ISO 13485-certified Quality Management System, each lot of Logix Smart™ ABC kit is tested against predetermined specifications to ensure consistent product quality.

TECHNICAL ASSISTANCE

For technical assistance, please contact our Technical Support:

- Website: **http://codiagnostics.com/contact/**
- Email: **info@codiagnostics.com**
- Phone: 801-438-1036 ext. 04

REFERENCES

Centers for Disease Control and Prevention. (2017, Oct 27). CDC Laboratory Training: Good Laboratory Practices for Molecular Genetics Testing. Retrieved Mar 5, 2019, from CDC: **https://www.cdc.gov/labtraining/training-courses/good-lab-practices-molecular-genetics-testing.html**
Poritz, M. A., & Ririe, K. M. (2014). Getting Things Backwards to Prevent Primer Dimers. The Journal of Molecular Diagnostics, 16(2), 159-162. doi:10.1016/j.jmoldx.2014.01.001 Satterfield, B. (2014, Mar). Cooperative primers: 2.5 million-fold improvement in the reduction of nonspecific amplification. The Journal of Molecular Diagnostics, 16(2), 163-173. doi:10.1016/j.jmoldx.2013.10.004 Viana, R. V., & Wallis, C. L. (2011). Good Clinical Laboratory Practices (GLCP) for Molecular Based Tests Used in Diagnostic Laboratories. In D. I. Akyar, Wide Spectra of Quality Control (pp. 29-52). InTech. Retrieved from **http://www.intechopen.com/books/wide-spectra-of-quality-control/**goodclinical-laboratory-practice-gclp-for-molecular-based-tests-used-in-diagnostic-laboratories

TRADEMARKS AND DISCLAIMERS

LEGEND OF PACKAGE SYMBOLS

REF	Catalog number
LOT	Batch Code
CAP	Cap color
COMP	Component
CONT	Content/Volume
NUM	Number
	Use-by-date
	Contains sufficient for X tests/ reactions X = 25 sample size X = 100 regular size
	Protect from light
	Temperature limit
	Consult Instructions for Use
	Non-Sterile product – Do not sterilize
	Manufacturer
RUO	Research Use Only

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Documents / Resources

	[CO-DIAGNOSTICS Logix Smart ABC Test Kit](#) [pdf] User Manual Logix Smart ABC Test Kit, Smart ABC Test Kit, ABC Test Kit, Test Kit, Kit

References

- Contact Us | Co-Dx - Co-Diagnostics, Inc.
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- Instructions For Use - Co-Diagnostics, Inc.
- codiagnostics.com/resources/safety-data-sheets/
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