UC-TIB-Respi-BAC-1 (*S.pneu/B.pert/B.para/H.inf*)

Cat.-No. 40-0409-UC-R Roche SAP No. 10 175 362 001

Storage at Arrival: 2-8°C in the dark
Do not freeze the reagents.

1. Content, Storage and Expiry

1 Vial red cap primer/probes (PSR) for 192 PCR reactions, 0.6 mL volume
2 Vials green cap Positive Control, 0.4 mL volume each

The product is shipped at ambient temperature.

Storage:

Upon arrival, store kits cooled (2°C to 8°C). Do not freeze reagents. Store in the dark.
Kits are stable for 18 months post-production; each vial contains the lot-specific expiry date.

On-board Stability:
The **cobas® omni** utility channel Reagent kit cassette filled with PCR Mix (containing Master Mix Reagent-2 (UC-MMx-R2) and **UC-TIB-Respi-BAC-1** PSR) can be stored refrigerated for up to 90 days from first use and used up to 40 times.

2. Recommended Additional Materials (Not Provided)

Materials and consumables:

For a detailed list of materials and consumables required for **cobas®** 5800 and **cobas®** 6800/8800 Systems refer to General Guidance for the use of **UC-TIB-Kits** with the **cobas® omni** utility channel on the **cobas®** x800 Systems Version 1.0 or higher.

Instrumentation and software:

Equipment	Roche P/N
cobas® 5800 System	08707464001
cobas® 6800 System (Option Moveable)	05524245001 and 06379672001
cobas® 6800 System (Fix)	05524245001 and 06379664001
cobas® 8800 System	05412722001
Sample Supply Module	06301037001
TWN3 Legic NFC USB (RFID Reader/Writer)	07450460001
External PC with remote connection provided by the customer	N/A
Barcode Printer	N/A

Instrument	Software	Version
cobas® 5800 Systems	**cobas®** 5800 software	1.0 or higher
External computer with remote connection (Remote User Interface, RUI)	**cobas® omni** CDC file creator, Navify	1.0 or higher
	cobas® omni utility channel optimization tool	5.1 or higher
cobas® 6800/8800 Systems	**cobas®** 6800/8800 software	1.4 or higher
External computer with remote connection (Remote User Interface, RUI)	**cobas® omni** utility channel tool	3.4 or higher
	cobas® omni utility channel optimization tool	4.1 or higher

Instructions:

Document	Version
cobas® omni utility channel User Assistance	4.4 or higher
cobas® omni utility channel for **cobas®** 5800 System User Assistance	1.0 or higher
General Guidance for the use of UC-TIB-Kits with the **cobas® omni** utility channel on the **cobas®** x800 Systems (https://elabdoc.roche.com)	1.0 or higher

3. Summary

This product detects *Streptococcus pneumoniae, Bordetella pertussis/holmesii, Bordetella parapertussis/ bronchiseptica and Haemophilus influenzae* DNA.

4. Principle

Bacterial DNA is analyzed by a real-time PCR, that amplify a 75 bp long fragment of lytA gene from *Streptococcus pneumoniae* analyzed with a Coumarin labeled probe in channel 1, a 69 bp long fragment of IS481 gene from *Bordetella pertussis/holmesii* analyzed with a FAM labeled probe in channel 2, a 80 bp long fragment of IS1001 gene from *Bordetella parapertussis/bronchiseptica* analyzed with a HEX labeled probe in channel 3 and a 137 bp long fragment of siaT gene from *Haemophilus influenzae* analyzed with a JA270 labeled probe in channel 4.

5. Specification

This assay detects at least 92 genome equivalent copies or less per mL for *Streptococcus pneumoniae*, 68 copies or less per mL for *Bordetella pertussis*, 82 copies or less per mL for *Bordetella parapertussis* and 105 copies or less per mL for *Haemophilus influenzae.* Determined by PROBIT with a hit rate of 95 % (LoD for 200 µL sample volume spiked with plasmid DNA).

6. Sample Material

Typical specimens are respiratory sample, like nasal, oro- or nasopharyngeal swabs. For best results, follow the General Guidance for the use of **UC-TIB-Kits** with the **cobas® omni** utility channel on the **cobas®** x800 Systems.

7. Assay Preparation/Procedure

Combine 10.0 mL UC-MMx-R2 with 600 µL PSR-Mix and transfer 9.7 mL of the prepared Master Mix through the bottom septum into the empty container in row 2.

Note: For detailed instructions see also General Guidance for the use of UC-TIB-Kits with the **cobas® omni** utility channel on the **cobas®** x800 Systems.

7.1 Instrument Programming

The PCR profile follows the default programming for the **cobas® omni** utility channel, as described in Table 1. The recommended sample volume and channel programming are described in Table 2.

Table1: Recommended PCR profile

PCR Profile				
Phase	**Step**	**Temperature (°C)**	**Hold time (s)**	**Cycles**
UNG-Treatment*	1	50	120	1x
	2	94	5	
Pre-PCR	1	55	120	x 1
	2	60	360	
	3	65	240	
1st Measurement	1	95	5	x 5
	2	55	30	
2nd Measurement	1	91	5	x 45
	2	58	25	

*) predefined for **cobas®** 6800/8800 Systems, the step is not visible in the cobas omni Utility Channel Tool

Table 2: Parameters for **UC-TIB-Respi-BAC-1**

Pipetting Profile and detection profile				
Sample material		**Processing volume (µL)**		
U_simple sample		200-800		
U_sample with swab		400		
Samples				
Channel	**Target Name**	**RFI min**	**Ct min**	**Ct max**
Channel 1	*Streptococcus pneumoniae*	1.5	0	50
Channel 2	*Bordetella pertussis/holmesii*	1.5	0	50
Channel 3	*Bordetella parapertussis/bronchiseptica*	1.5	0	50
Channel 4	*Haemophilus influenzae*	1.5	0	50
Channel 5	IC	2.5	0	50

7.2 Workflow Overview

- Do not use **cobas**® **omni** utility channel reagent kit, **cobas**® buffer negative control kit, **UC-TIB-Respi-BAC-1** or **cobas**® **omni** reagents after their expiry dates.
- Do not reuse consumables which are designed for single use only.

Table 3: Workflow Overview

Step	Action	Required material	Reference Document
1	Define Tests Ordering	**cobas**® x800 Systems	**cobas**® **omni** utility channel User Assistance **cobas**® **omni** utility channel for **cobas**® 5800 System User Assistance
2	Prepare & Load Reagent Cassette		
3	Load Reagents and Consumables		
4	Prepare Samples and Control		
5	Start Run		
6	View Results		
7	Unload Consumables		

8. Prepare Samples and Control

One positive control ((+)_Ctrl) has to be performed as a sample in each run and for each new reagent cassette.

► Refer to the **cobas**® x800 Systems User Assistance for instructions on how to identify sample tubes with barcodes.

Prepare the secondary tube for the **Respi-BAC-1** (+)_Ctrl with the corresponding barcode as described below:

- Vortex the DNA control vial
- Transfer 20 µL of the DNA control into 1 mL of negative matrix (e.g. water, PBS or known negative specimen) and mix by vortexing

Please note, a negative control (**cobas**® buffer negative control kit, Roche P/N 09 051 953 190) is automatically performed with each run on **cobas**® x800 Systems.

9. Result Analysis

9.1. Quality Control and Run Validity of the Results

The negative and the (+)_Ctrl validate the run while the IC validates each sample. To determine this validity, interpret the results from the controls as described in Table 4 below.

Table 4: Run and Reaction Validity Interpretation

Validity	Control	Valid	Invalid	Validation
Run	negative control	Indicated as "Yes" in Overall Result column	Indicated as "Invalid" in Overall Result column ► All samples of the run must be retested	**cobas**® x800 Systems
	(+)_Ctrl	Ct value indicated in each Target column	Indicated as "Negative" in the Target column 1, 2, 3 or 4 ► All samples of the run must be retested	Operator
Sample	IC	Indicated as "Yes" in Valid column	Indicated as "No" in Valid column AND Target 1, 2, 3 or 4: Negative ► Invalidated sample must be retested	Operator

9.2. Interpretation of the Results

If both the run and sample are valid, the interpretation of the results for each target is based on the results provided by the **cobas**® x800 Systems as described in Table 5.

Invalid results for one or more target combinations are possible and are displayed on the **cobas**® x800 system for each individual channel. Samples with invalid results must be retested. If the target result remains invalid, a new sample must be run.

Table 5: Sample Result Interpretation

Channel 1	Channel 2	Channel 3	Channel 4	Interpretation
S.pneumoniae Ct Value	*B.pertussis* Negative	*B.parapertussis* Negative	*H.influenzae* Negative	Target signal detected for ***Streptococcus pneumoniae***
S.pneumoniae Negative	*B.pertussis* Ct Value	*B.parapertussis* Negative	*H.influenzae* Negative	Target signal detected for ***Bordetella pertussis/holmesii***
S.pneumoniae Negative	*B.pertussis* Negative	*B.parapertussis* Ct Value	*H.influenzae* Negative	Target signal detected for ***Bordetella parapertussis/bronchiseptica***
S.pneumoniae Negative	*B.pertussis* Negative	*B.parapertussis* Negative	*H.influenzae* Ct Value	Target signal detected for ***Heamophilus influenzae***
S.pneumoniae Negative	*B.pertussis* Negative	*B.parapertussis* Negative	*H.influenzae* Negative	No target signal detected for **UC-TIB-Respi-BAC-1**
Invalid	Invalid	Invalid	Invalid	Result for **UC-TIB-Respi-BAC-1** is invalid

10. Precautions and Warnings

- For research use only, before using this product, read the operator / safety instructions in the instruments operator's manual.
- General precautions for the handling of samples and generic laboratory materials are required.
- Use personal protective equipment such as laboratory coats, gloves and eye protection when handling samples, consumables and reagents.
- All materials of human origin and related waste must be considered potentially infectious.
- Do not eat, drink or smoke in the laboratory. Do not pipette by mouth.
- The use of disposable filter tips is mandatory. Dispose of the unused reagents and inactivate waste materials according to the current local guidelines.

11. References

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12. Certificate of Origin

Product is not from human, animal or plant origin. Country of Origin: Germany

13. Manufacturer and Contact Details

Report device observations, deviations and problems to your local Roche representative. Please report lot number(s) and a brief description.

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14. Version History

Notes in red mark: events requiring changes in procedures.

v1.0	Release version	2024-01-18
v1.1	5. Specification: LoD specified	2024-02-29
v1.2	Table 1, UNG-Treatment step programming for **cobas**® 5800 system	2024-03-25