qPCR –
Bacteria, Resistance, Fungi, IPC

cube dx
hybCell technology

January 2023
V005

Eluate from the GINA kit

20µl each

"Ready-to-use" PCR - Master mix

Colours:

Red – Bacteria (16S)
Green – Fungi (28S)
Yellow – Resistance
Blue – IPC

→ ***Close PCR - Master mixes***

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Load PCR - device

→

PCR - protocol:

→ ***Start PCR***

94°C 1 min.

<u>41 Cycles:</u>
94°C 5 sec.
56°C 10 sec. *+plate read*
72°C 30 sec.

72°C 1 min.

<u>Melt (only qPCR):</u>
75°C to 94°C, increment 0,5°C steps
10 sec. *+plate read*

25°C (hold)

Fluor: SYBR Green

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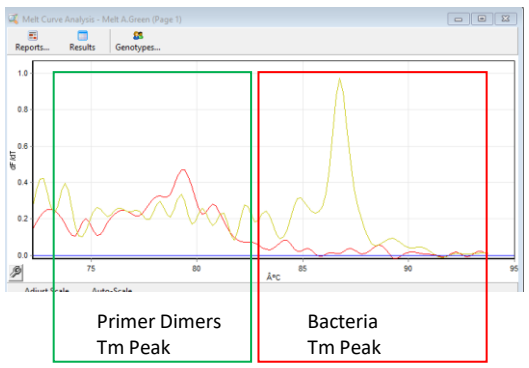
<u>Validated Thermal cyclers</u>
- Rotor-Gene
- CFX96
- Quantstudio 3/5
- Tpersonal Thermocycler (Biometra)

PCR interpretation (only qPCR)



1. *Optional: Check the Internal Process Control (IPC) - PCR:*
   - *Valid, if Cq < 32*
   - *Discard test results, if IPC is invalid*
2. *Interpretation of the Cq and melt curves require laboratory expertise and experience in working with blood or BAL samples.*
3. *Thermal cyclers may differ in their thermal characteristics, therefore the optimization of the temperatures in the protocol may be recommended (for validated devices) if the results are undesirable.*

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Storage: ***-25° to -18°C***

Contact: Cube Dx GmbH, Westbahnstraße 55, A-4300 St. Valentin/Austria, +43 7435 58193 info@cubedx.com, www.cubedx.com

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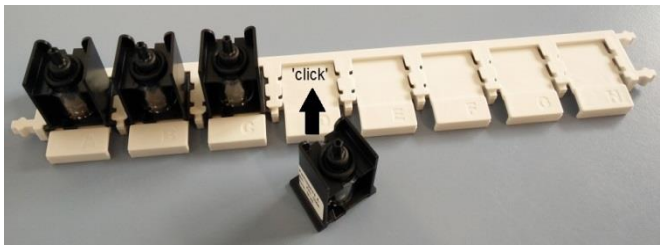
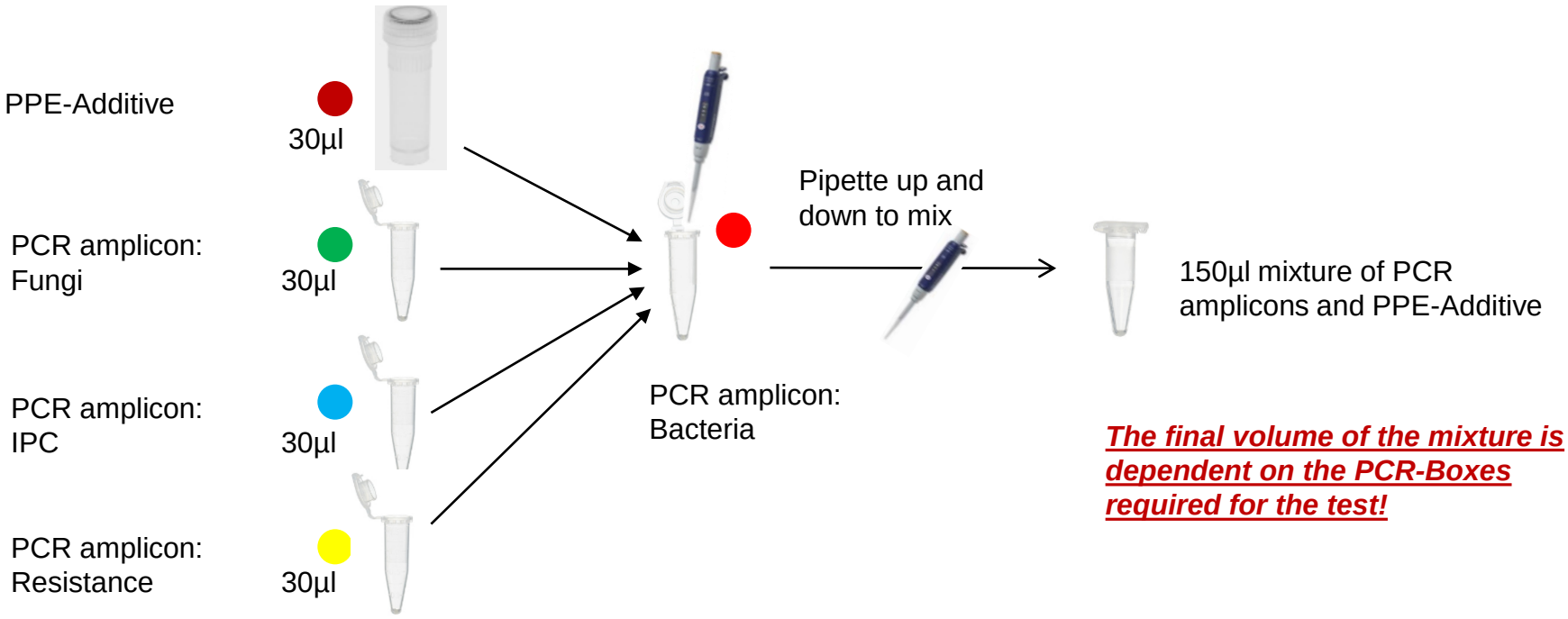
Unpack hybcell

Insert into rack: "click"



	Bac. PCR	Res. PCR	Fun. PCR	IPC PCR
hybcell Bacteria	•	•		•
hybcell Fungi			•	•
hybcell Pathogens	•	•	•	•

Combine all the desired amplicons (30µl each) into any one of the amplicon tubes. Pipette 30µl of the PPE-Additive into the mixing tube.



The final volume of the mixture is dependent on the PCR-Boxes required for the test!

Fill the hybcell with the (positive) PCR-amplicon and PPE-Additive mixture

Pipette the entire volume gently and at once!

Insert the tip of the pipette deeply into the hybcell!

Try not to wet the hybcell's inside margins!

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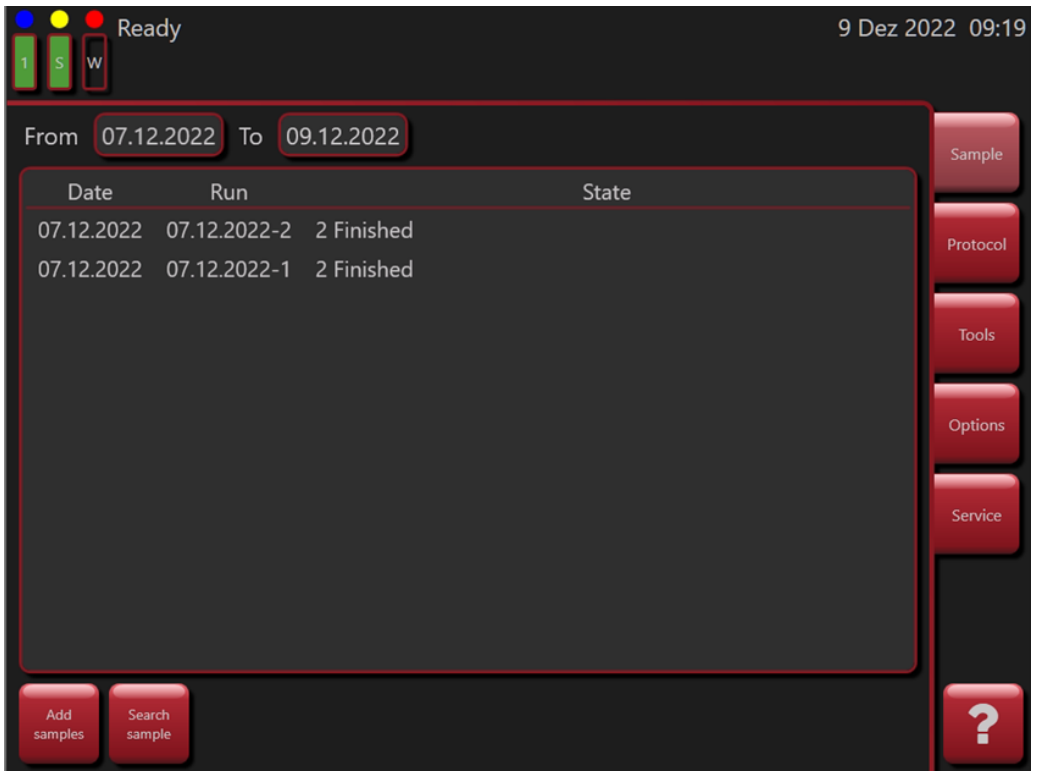
Create a run and start



1. ***Create new sample (“Sample” screen)***
2. ***Insert data (sample ID, hybcell ID)***
3. ***Select samples and start run (“Sample” screen)***
4. ***Insert the rack (Barcode facing inwards) and confirm***

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