




LINA + qPCR – Bacteria, Resistance, Fungi



Positive blood culture
2µl

BAL
20µl

LINA → **Invert several times or vortex**




IPC 20µl (Optional)

Spin down the tube before use!






LINA → **Invert several times or vortex**



From the LINA – solution

20µl per reaction

“Ready-to-use” PCR- master mix






Colours:

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

→ **Close PCR-master mixes**

Load PCR - device

Validated Thermal cyclers

- Rotor-Gene
- CFX96
- Quantstudio 3/5
- Tpersonal Thermocycler (Biometra)

PCR - protocol:

95°C 2 min. → **Start PCR**

45 Cycles:

- 95°C 10 sec.
- 56°C 10 sec.
- 72°C 30 sec.

+ plate read

75°C 1 min.

Melt (only qPCR):

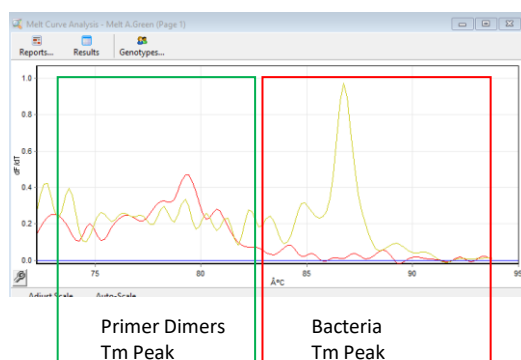
75°C to 95°C, increment 0,3°C steps
10 sec. + plate read

25°C (hold) Fluor: SYBR Green

PCR interpretation (only qPCR)

The conditions in the table below must be met for a PCR result to be identified as positive.

See the IFU, Pathogens xB for more information on PCR interpretation.



	Ct-Value	Melting Temperature
Bacteria	< 34	80°C to 90°C
Fungi	< 40	80°C to 94°C
Resistance	< 34	80°C to 90°C
IPC	< 34	86°C ± 1°C

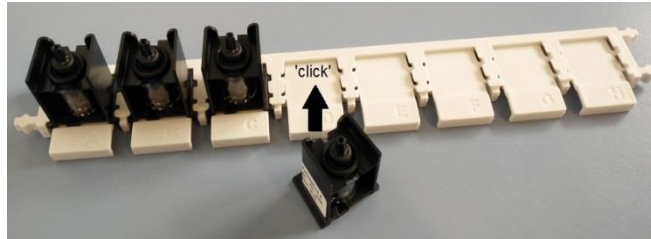
Storage: -15° to -25°C

hybcell – Bacteria, Fungi or Pathogens

Unpack hybcell



Insert into
rack: "click"



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



Combine up to maximum 3 of the desired amplicons (30µl each) into any one of the amplicon tubes.
Pipette 30µl of the PPE-Additive into the mixing tube.

PPE-Additive



30µl

PCR amplicon:
Fungi



30µl

Pipette up and
down to mix



PCR amplicon:
Bacteria



40µl



100µl mixture of PCR
amplicons and PPE-Additive



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

Different examples of amplicon combinations suitable for the loading of the hybcell

Results of PCR				Amplicons transferred into hybcell
Bacteria	Fungi	Resistance	IPC	
pos.	pos.	pos.	pos.	Bacteria + Fungi + Resistance
pos.	pos.	neg.	pos.	Bacteria + Fungi
pos.	neg.	pos.	pos.	Bacteria + Resistance
neg.	pos.	neg.	pos.	Fungi + IPC

hybcell – Bacteria, Fungi or Pathogens

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

Pipette the entire volume
gently and at once!

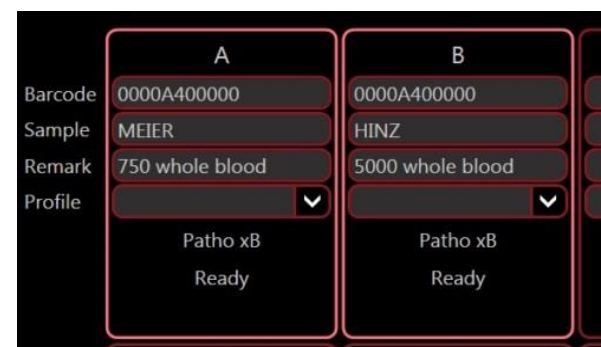
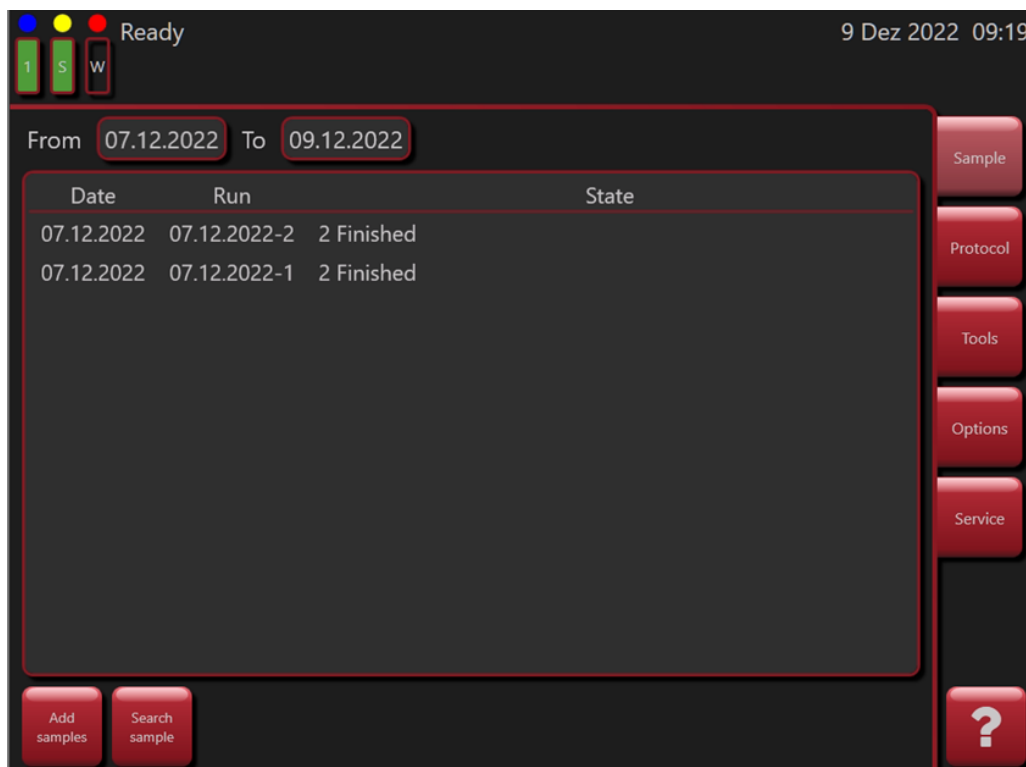


Insert the tip of the
pipette deeply into the
hybcell!

Try not to wet the
hybcell's inside
margins!

Once finished, close the
hybcell with the lid.

Create a run and start



1. Create a 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

