Guidelines for PCR Result-Interpretation

Pathogens xB on PSS geneLeadVIII

PCR Box Bacteria 48rx-s

PCR Box Fungi 48rx-s

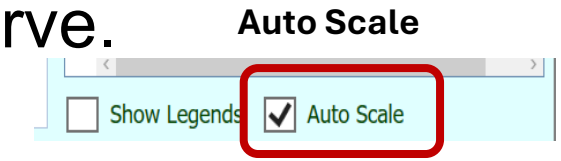
V 01, January, 2026.

Objectives and Possibilities

- Inhibition of PCR is indicated
  - Conclusions on the quality of samples and the sample preparation process can be drawn
- Most negative samples (not detected) can be sieved out
- Select samples for testing with hybcell (ID)
- Ct-values and melting peaks help to estimate the initial concentration of targets in the sample
  - Supporting the decision of clinical relevance (vs. contamination)

Workflow

1. Check the appearance of the amplification and melting curve.
   1. Check if the “Auto Scale” box is active (otherwise activate)
   2. Assess the appearance and shape of the amplification and melting curves
   3. Verify the calculated Cts and melting points (approximation)



2. Evaluate if any inhibition might have taken place → repeat extraction+PCR
   - E.g. no positive amplification neither for target nor IC, or an amplification curve “going down” or an amplification curve with no exponential increase…

3. Evaluate Ct-values and melting peaks according to following rules / examples
   1. Determine, which samples have tested negative (=not detected) for the target
   2. Select all other samples (not negative) for further testing with hybcell

Suggested cut-off values (patient samples)

	Amplification Curve	Melt Curve		
	Ct	Tm1 (background)	Tm2 (IC)	Tm3 (target)
Bacteria				
Test with hybcell	**< 37**	(< 79.5 °C)	(79.5 – 81.5 °C)	**> 83 °C**
Not detected	**> 37**	(< 79.5 °C)	**79.5 – 81.5 °C**	--
Fungi				
Test with hybcell	**< 37**	(< 80.0 °C)	(80.0 – 81.8 °C)	**> 83 °C**
Not detected	**> 37**	(< 80.0 °C)	**80.0 – 81.8 °C**	--

Grey Boxes indicate mandatory criteria.

Ct ... Cycle threshold (cycle number)
Tm ... Melting temperature (°C)

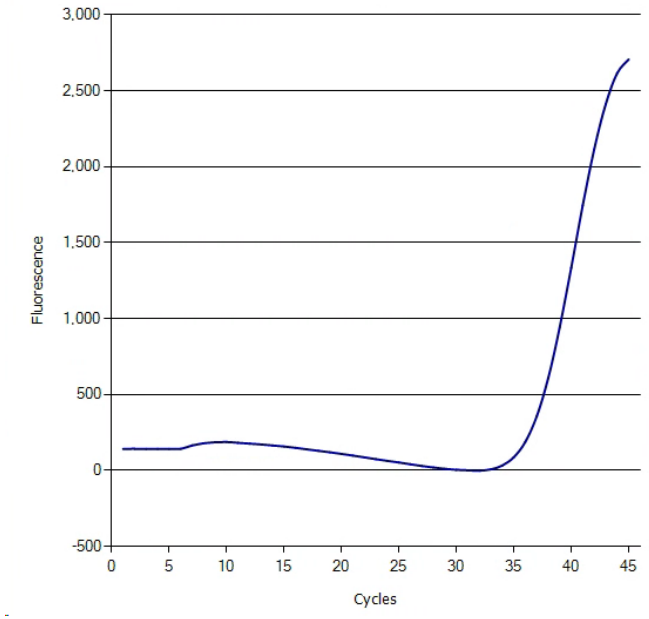
IC … Internal control
( ) ... could be missing at low Ct

Experiences

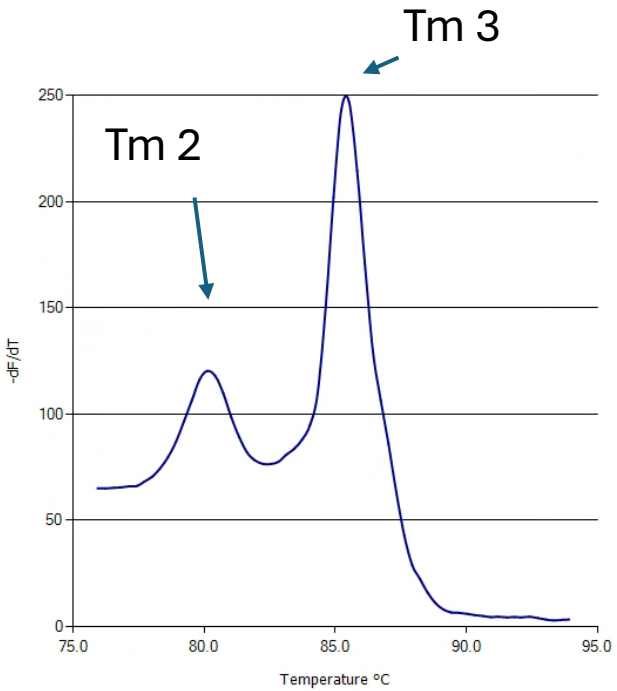
1. A “high” melting peak (Tm3) is the best indication for positive amplification of the target
    1. If the peak at Tm3 is higher than the one at Tm2, the sample should be tested with hybcell anyway (even if Ct is slightly higher than threshold).
    2. If the peak at Tm3 is lower than the one at Tm2, the amplification is likely caused by contamination
2. Ct-values and melting peaks might shift dependent on..
    1. ..tested samples and used preparation methods
    2. ..used lot of PCR-boxes

Bacteria – example of positive result

Amplification Curve



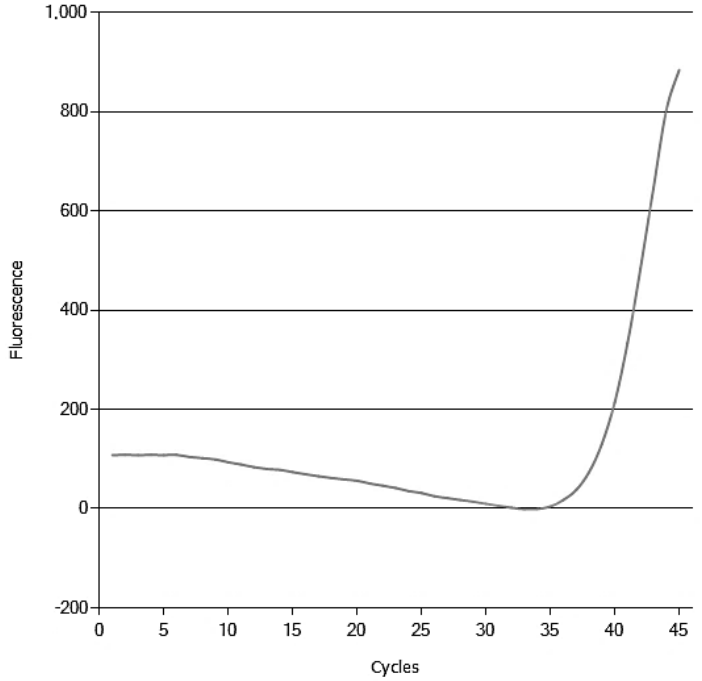
Melt Curve



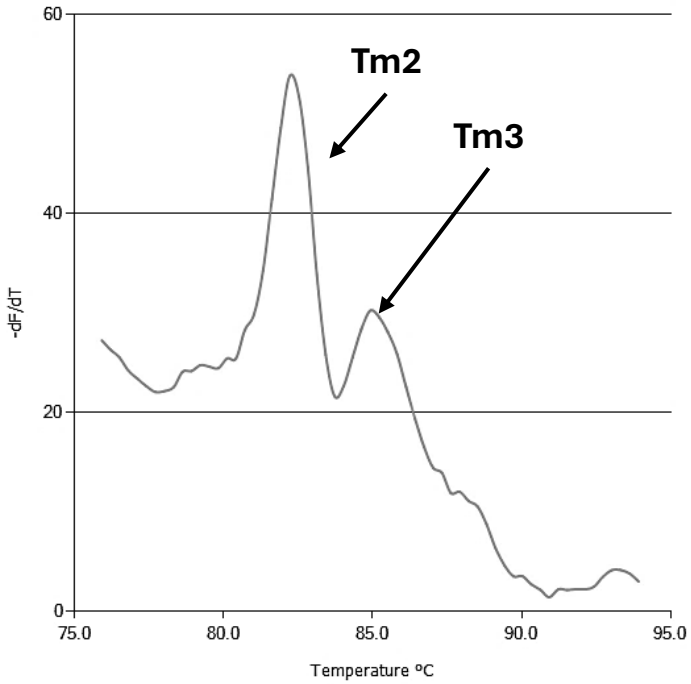
	Ct	Tm1	Tm2	Tm3	Conclusion:
	36.08	-	80.2°C	85.4 °C	
Interpretation:	Positive Ct (< 37)	No background	IC peak – successful PCR amplification	Target	test with hybcell

Bacteria – example of negative result

Amplification Curve



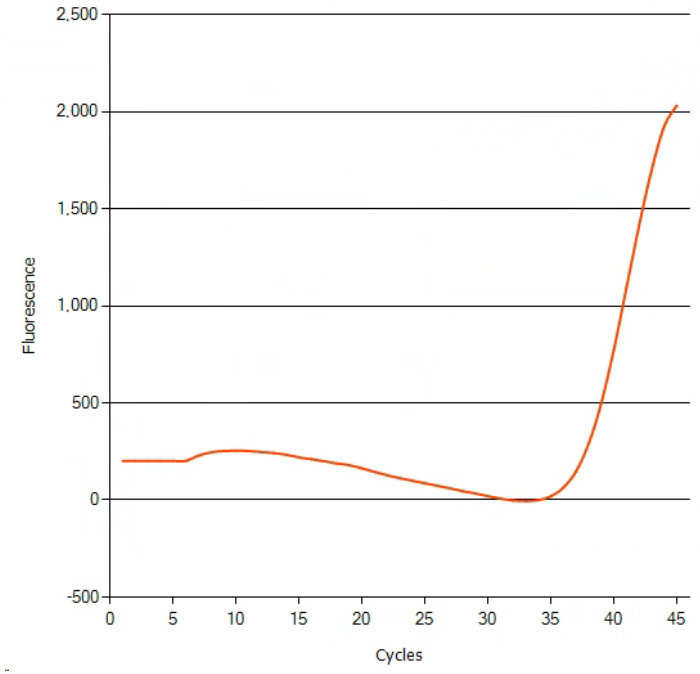
Melt Curve



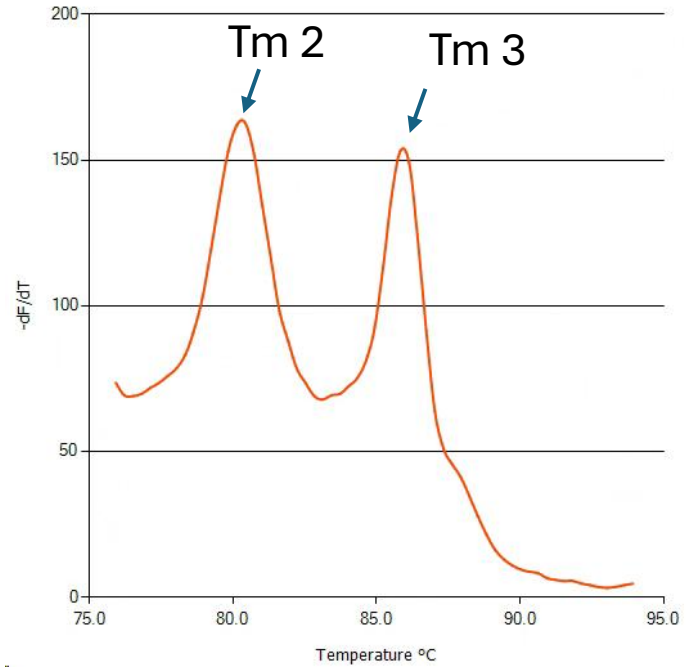
	Ct	Tm1	Tm2	Tm3	Conclusion:
	39. 8	--	81.5 °C	85.1 °C	
Interpretation:	Negative Ct (> 37)	No background	IC peak – successful PCR amplification	Possible contamination (target peak << IC peak)	**Not detected**

Bacteria – example of ambiguous result

Amplification Curve



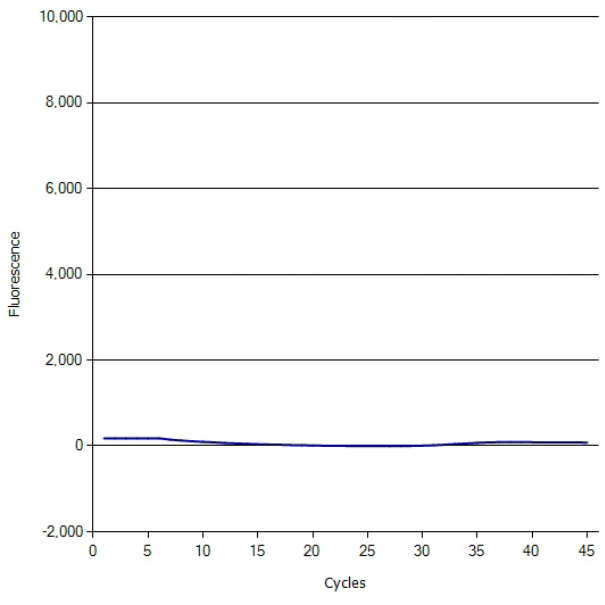
Melt Curve



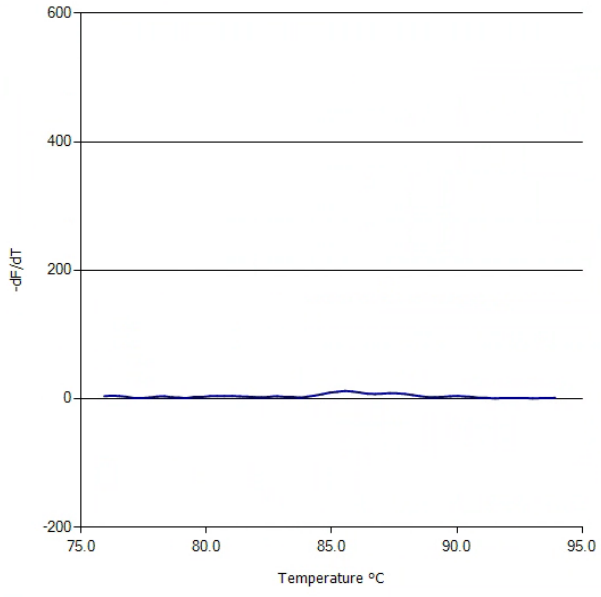
	Ct	Tm1	Tm2	Tm3	Conclusion:
	37.36	--	80.3 °C	86 °C	
Interpretation:	Negative Ct (> 37)	No background	IC peak – successful PCR amplification	Target ~ IC	**Doubtful, test with hybcell**

Bacteria – example of inhibited result

Amplification Curve



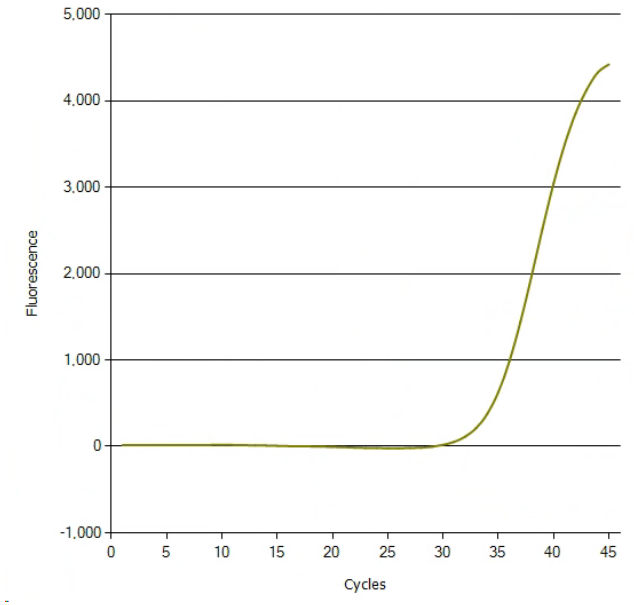
Melt Curve



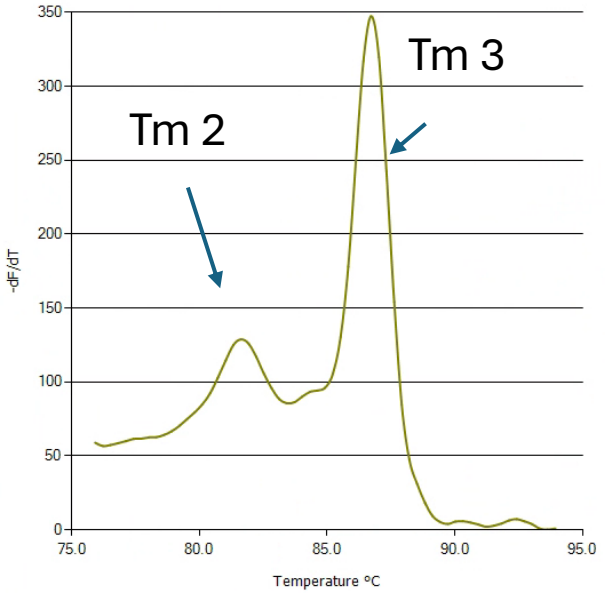
	Ct	Tm1	Tm2	Tm3	Conclusion:
	--	--	--	--	
Interpretation:	Negative Ct (> 37)	No background	No IC	No target	Repeat test (extraction+PCR)

Fungi – example of positive result

Amplification Curve



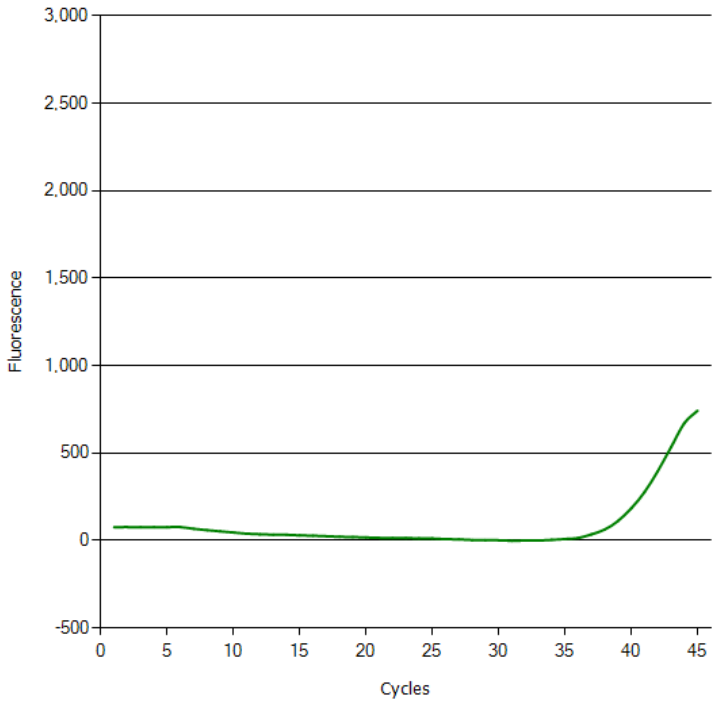
Melt Curve



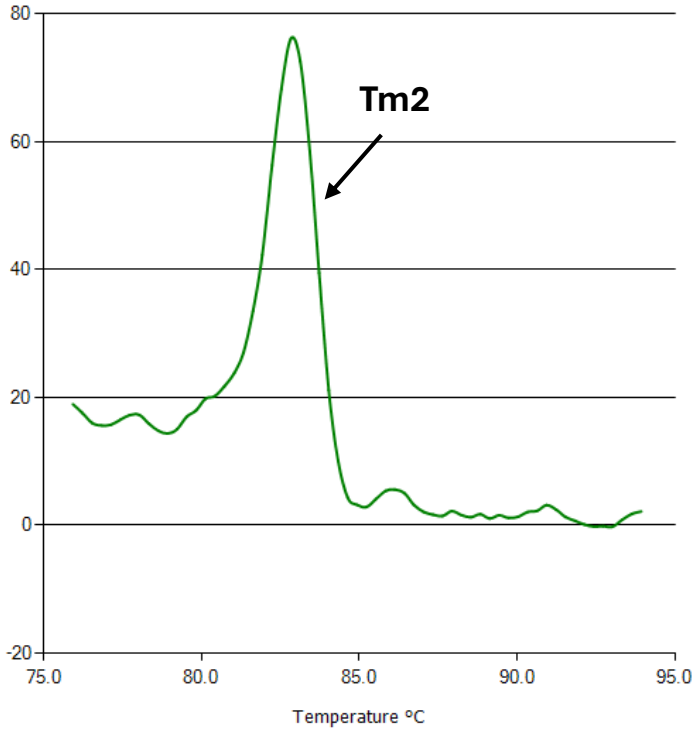
	Ct	Tm1	Tm2	Tm3	Conclusion:
	32.87	-	81.8 °C	86.7 °C	
Interpretation:	Positive Ct (< 37)	No background	IC peak – successful PCR amplification	Target peak >> IC	Test with hybcell

Fungi – example of negative result

Amplification Curve



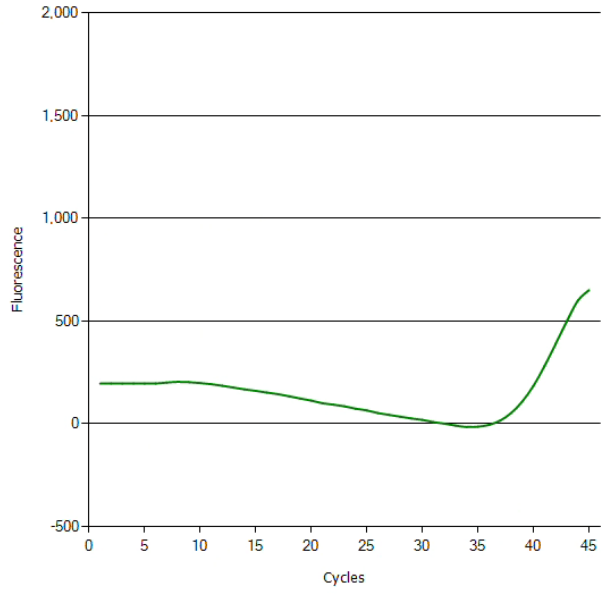
Melt Curve



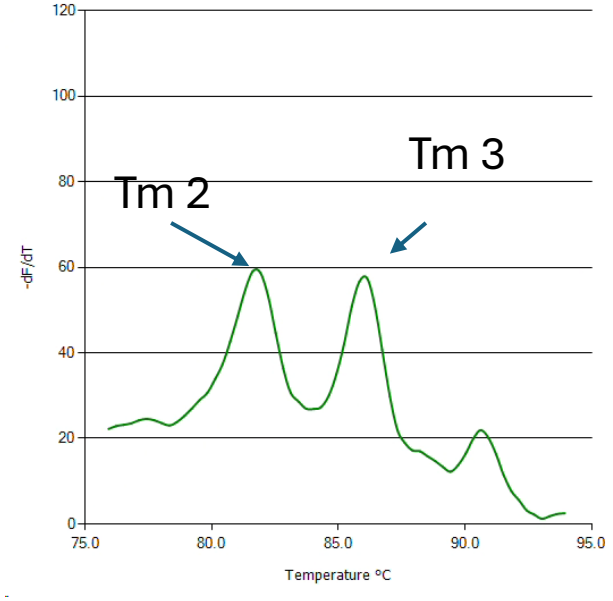
	Ct	Tm1	Tm2	Tm3	Conclusion:
	40.19	--	81.4 °C	--	
Interpretation:	Negative Ct (> 37)	No background	IC peak – successful PCR amplification	No contamination	**Not detected**

Fungi – example of ambiguous result

Amplification Curve



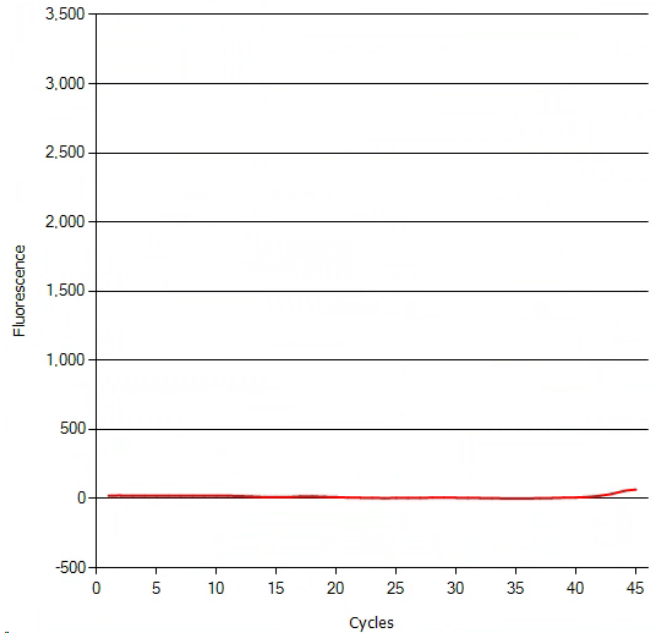
Melt Curve



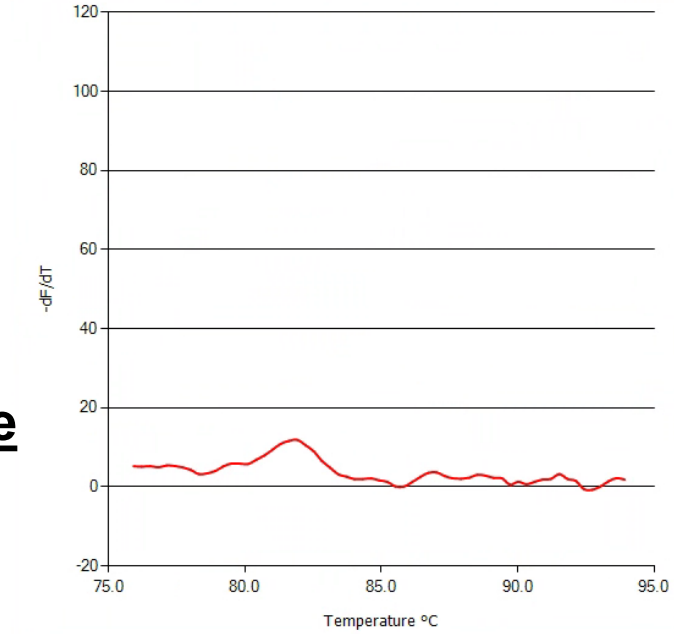
	Ct	Tm1	Tm2	Tm3	Conclusion:
	40.16	--	81.7 °C	86 °C	
Interpretation:	Negative Ct (< 37)	No background	IC peak – successful PCR amplification	Possible target (target peak ~ IC peak)	**Doubtful, test with hybcell**

Fungi – example of inhibited result

Amplification Curve



Melt Curve

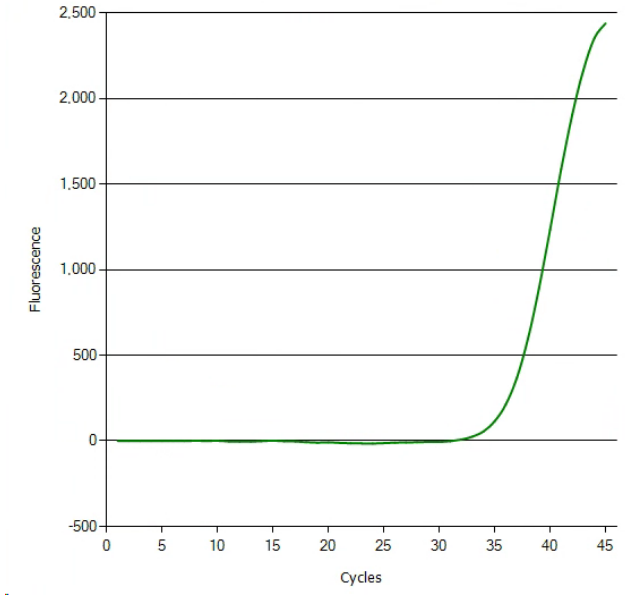


	Ct	Tm1	Tm2	Tm3	Conclusion:
	--	--	--	--	
Interpretation:	negative Ct (> 37)	No background	No IC	No target	Repeat test (extraction+PCR)

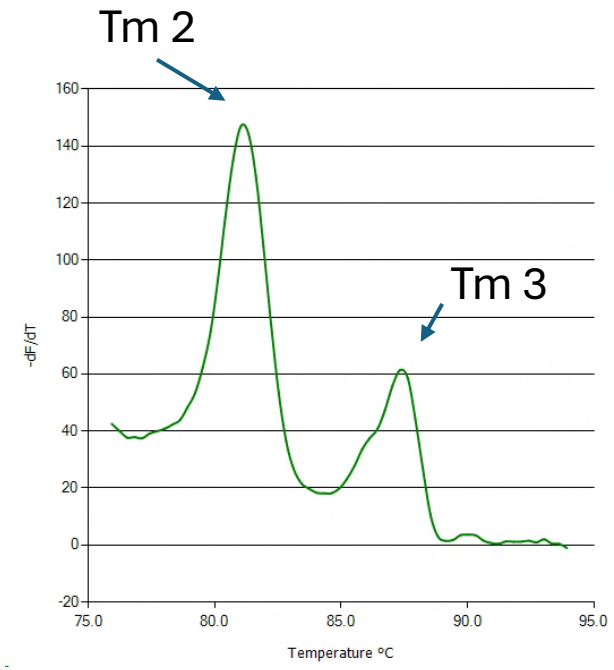
Using controls

Bacteria – NTC (from the kit box)

Amplification Curve



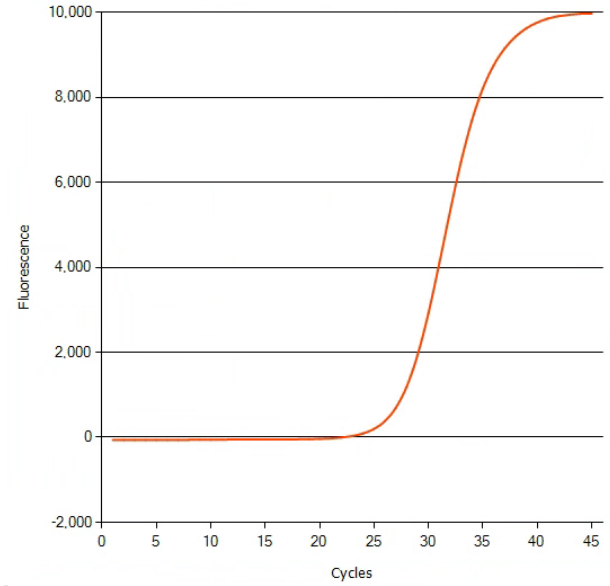
Melt Curve



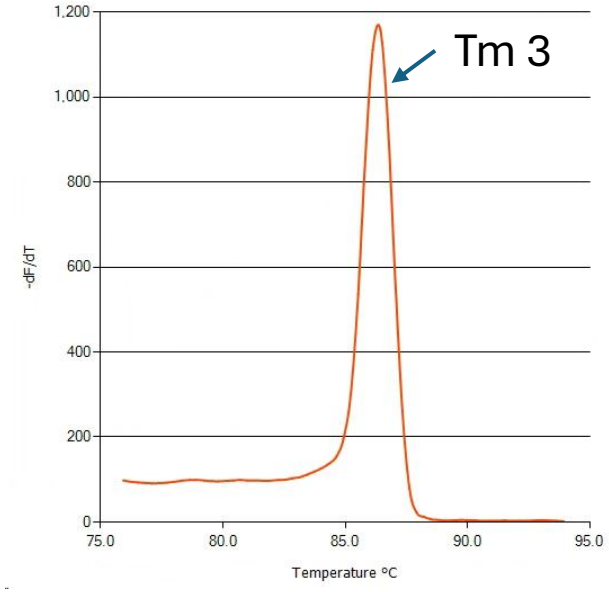
	Ct	Tm1	Tm2	Tm3	Conclusion:
	37.07	--	81.2°C	87.4°C	
Interpretation:	Negative Ct (> 37)	No background	IC peak – successful PCR amplification	Possible contamination (target peak < IC peak)	**Not detected**

Bacteria– Control DNA SA/CA

Amplification Curve



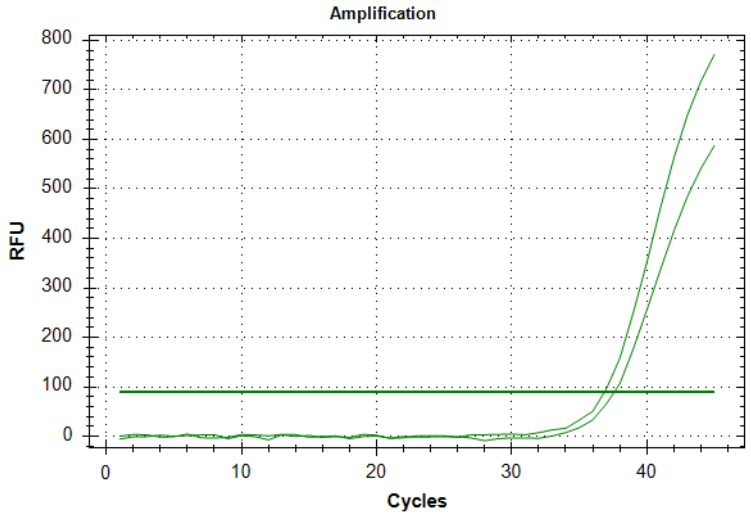
Melt Curve



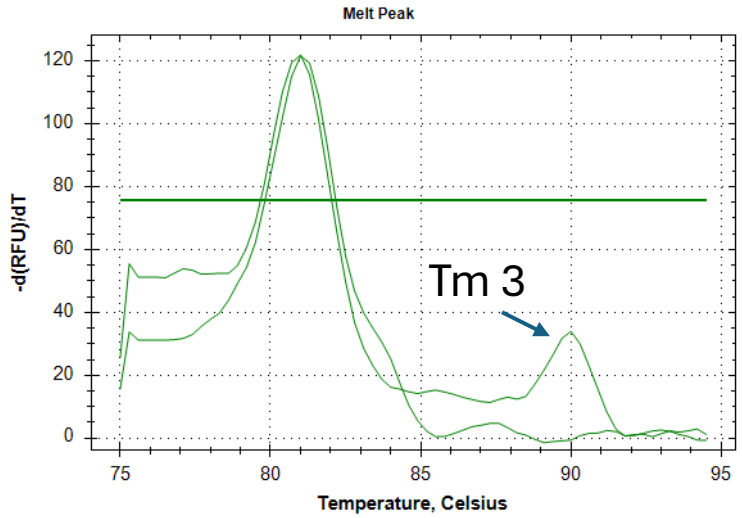
	Ct	Tm1	Tm2	Tm3	Conclusion:
	25.02	--	--	86.3 °C	
Interpretation:	positive Ct (< 37)	No background	No IC peak, abundance of target	Target	Test with hybcell (Staphylococcus aureus)

Fungi – NTC

Amplification Curve



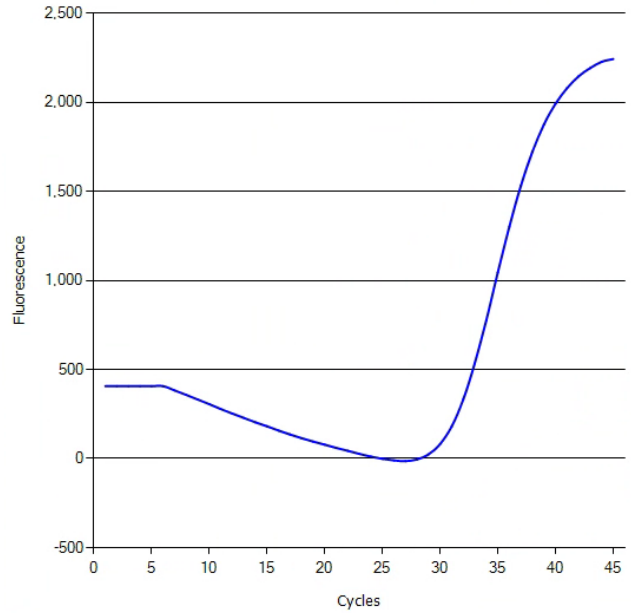
Melt Curve



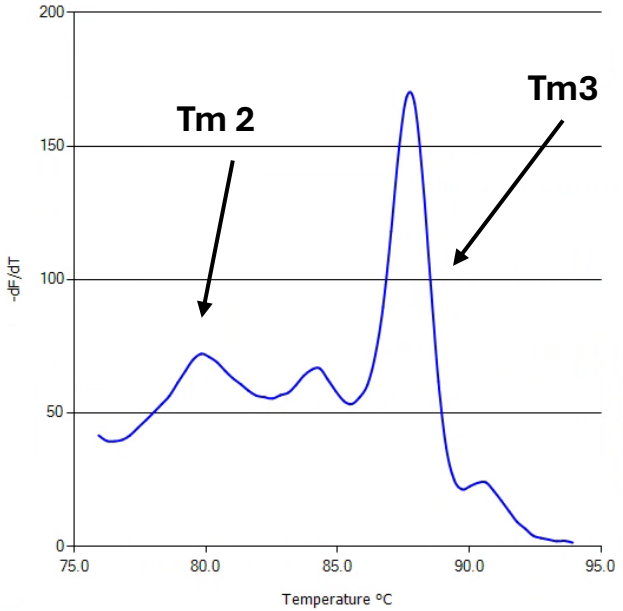
Lane	Ct	Tm1	Tm2	Tm3	Conclusion:
1	37.6	--	81 °C	--	
Interpretation:	negative Ct (> 37)	No background	IC peak – successful PCR amplification	No contamination	**Not detected**
2	36.9		81 °C	90°C	
	borderline	No background	IC peak – successful PCR amplification	Possible contamination (target peak < IC peak)	**Doubtful, test with hybcell**

Fungi – Control DNA SA/CA

Amplification Curve



Melt Curve

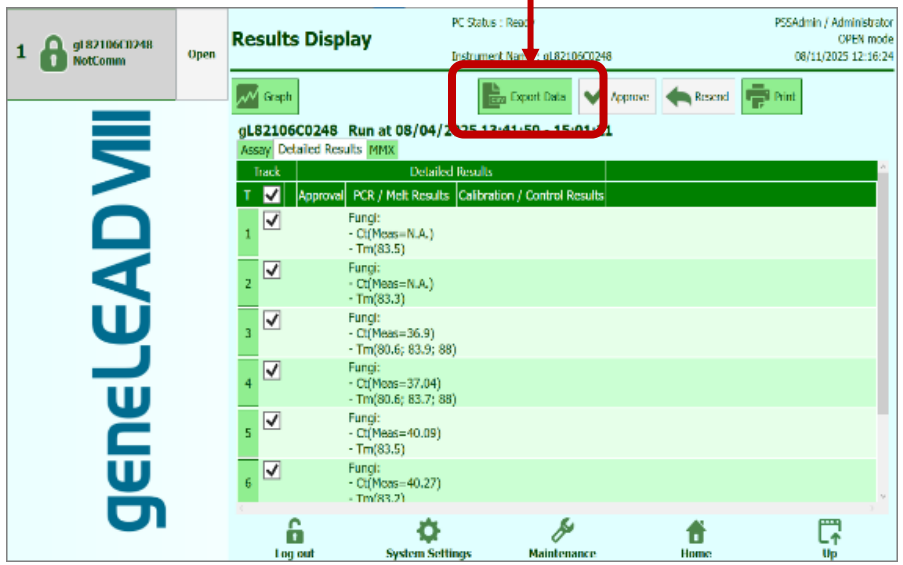


	Ct	Tm1	Tm2	Tm3	Conclusion:
	31	--	~80 °C	87.7 °C	
Interpretation:	Positive Ct (< 37)	No background	IC peak – successful PCR amplification	Target	Test with hybcell (Candida albicans)

What to do in case of invalid results?

Measures:

1. Repeat the experiment
2. Test PCR with **control DNA** (SACA)
3. Test extraction/PCR with **EPC** (withing sample)
4. Reduce sample volume
5. Ask for help via the **support form** (www.cubedx.com/en/support) – send the **exported run data** from PSS gLVIII (see below)



Possible reasons:

- No PCR mix pipetted
- No sample DNA added
- Wrong PCR protocol selected
- **Inhibition of PCR** (e.g. EtOH, hypochlorite...)
- Excessive Beads within eluate (PCR tube)
- Contamination/impurities (e.g. dust, hair, ...)
- Instrument errors (pipetting, beads)
- ...

Use of “Auto Scale”

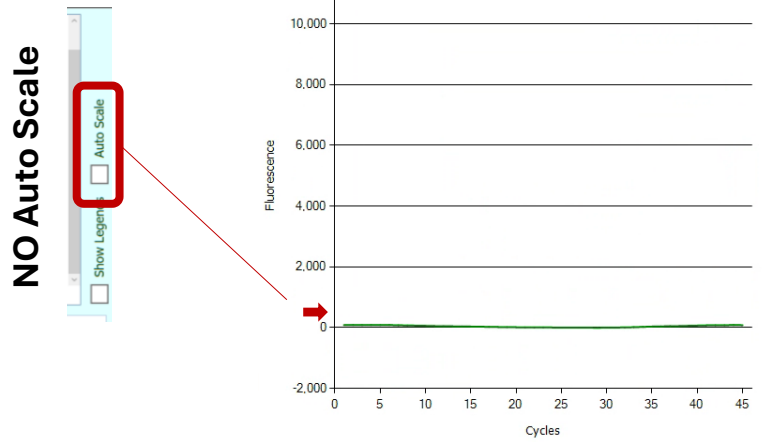
Comparison results without and with Auto Scale

Note: Auto-scaling might change the appearance of flat curves into ascending, descending, or irregular curves (different scaling).

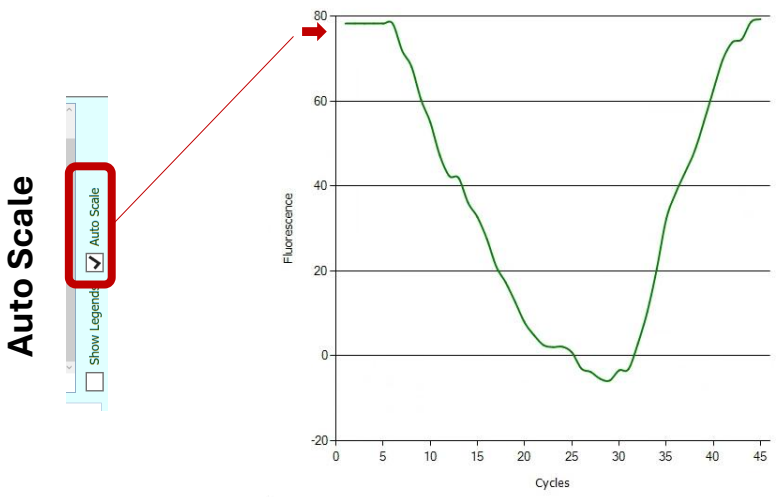
Always check the y-axis range of the amplification in case of questionable results and compare visualisation in both modes (auto scale on/off)!

Possible Amplification curves

NO Auto Scale



Auto Scale



Melting curves