

Important Note

QIAcuityDx® Four

Dear valued QIAGEN® customer,

Due to inherent variables associated with dPCR, you may occasionally notice two populations of partitions at low (baseline) fluorescence intensities in 1D scatterplots for certain multiplexed assay designs. This low intensity double population can be the result of carryover signals from neighboring channels, known as “cross talk,” which is a common characteristic in multiplex dPCR systems due to the overlap of the emission spectrum for some fluorophores.

When cross talk occurs, multiple bands can appear at low RFU values (in the example presented in Figure 1, <20 RFU, but can be dependent on probes concentration and quenching efficiency). Occasionally, the current automatic thresholding algorithm may identify the cross-talk signal as the “positive” partition population and set the threshold between the negative population and the cross talk population (well A1). In such cases, we recommend manually setting the threshold above the cross talk population (as shown in well A2) to ensure optimal performance.

We appreciate your attention to this detail and are confident that these insights will enhance your experience with the QIAcuityDx System.

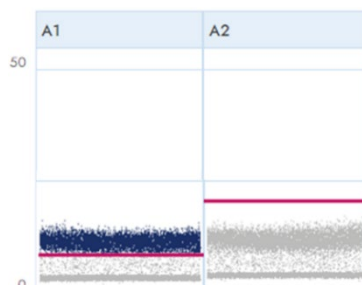


Figure 1. Cross talk with incorrectly applied threshold (well A1) and correctly applied threshold (well A2).

The following steps can be taken during assay design and run set up to minimize the impacts of cross talk and decrease the likelihood of incorrect auto-thresholding.

1. Select fluorophores that are not in neighboring fluorescence channels where possible (see www.qiagen.com/HB-3567 for supported fluorophores).
2. Optimize reaction composition (primer, probe, and MgCl₂ concentration) to reduce the effects of carryover signal in neighboring channel(s) (see www.qiagen.com/HB-3592 for reaction mix optimization guidance).
3. Optimize imaging parameters (Exposure and/or Gain) to avoid excessive signal which may result in low intensity signal carryover into neighbor channel(s) (see www.qiagen.com/HB-3567 for image parameterization guidance).

Should you wish to discuss this further, you can reach out to our QIAGEN Technical Services at support.qiagen.com

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