

The detection of multiple targets in the same dPCR reaction can conserve sample, shorten turnaround times, reduce pipetting errors, and maximize the data obtained from each run.

The **Digital LightCycler® dPCR System** can detect as many as 6 separate RNA or DNA targets in a single reaction while utilizing a separate channel as a process control.

Detect up to 6 unique targets in a single reaction

In the examples below, six separate genes are assayed simultaneously with different dyes, on six separate channels. Of these targets, four are frequently amplified in cancer, and two serve as a references for the nonamplified copy number (**Table 1**).

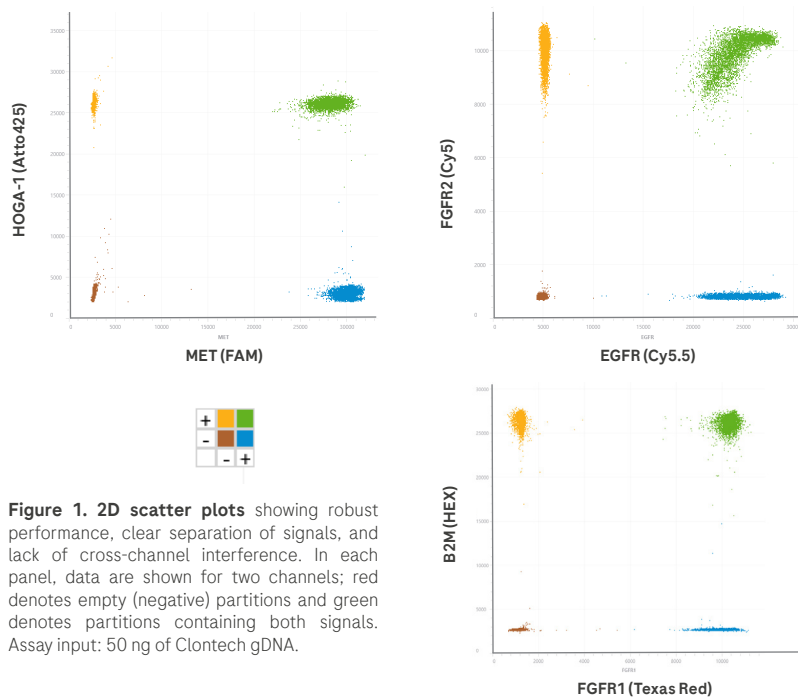


Figure 1. 2D scatter plots showing robust performance, clear separation of signals, and lack of cross-channel interference. In each panel, data are shown for two channels; red denotes empty (negative) partitions and green denotes partitions containing both signals. Assay input: 50 ng of Clontech gDNA.

Table 1. Channel assignment for targets of interest and reference targets of the example 6-plex assay. See **Figure 4** for a complete list of compatible dyes.

Gene of interest*	Optical Channel	Dye	Alteration in cancer
HOGA1	1	Atto425	Not expected (reference)
MET	2	FAM	Amplification
B2M	3	HEX	Not expected (reference)
FGFR1	4	Texas Red	Amplification
FGFR2	5	Cy5	Amplification
EGFR	6	Cy5.5	Amplification

*Genes are: B2M, β 2-microglobulin; EGFR, epidermal growth factor receptor; FAM, fluorescein amidite; FGFR1/2, fibroblast growth factor receptor 1/2; HEX, hexachloro-fluorescein; HOGA1, 4-hydroxy-2-oxoglutarate aldolase 1; MET, mesenchymal-epithelial transition.

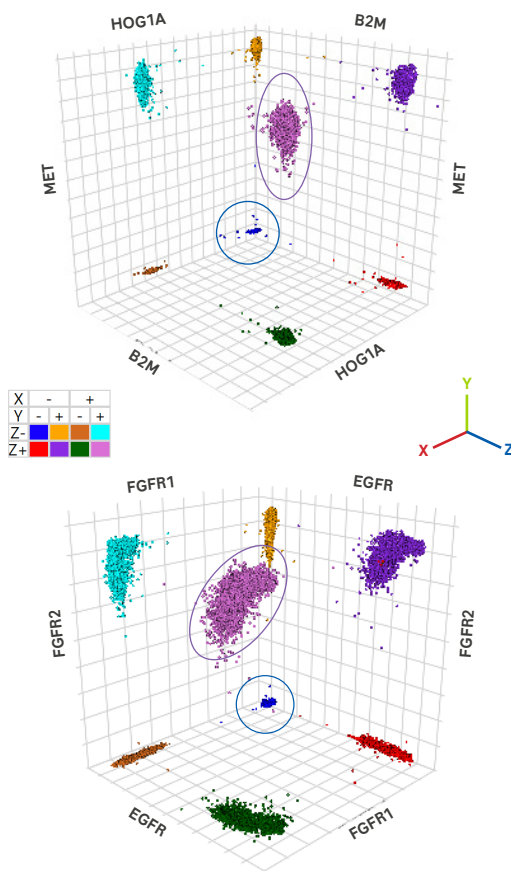


Figure 2. 3D scatter plots, each showing signal from 3 channels. Each “wall” in these charts shows signal from 2 targets, and is read similarly to the 2D charts shown in **Figure 1**. In each chart, the small, dark-blue cluster (blue circle) where 3 axes meet represents partitions that are negative for all 3 signals, and the light purple cluster (purple oval) that appears closest represents partitions that are positive for all 3 targets.

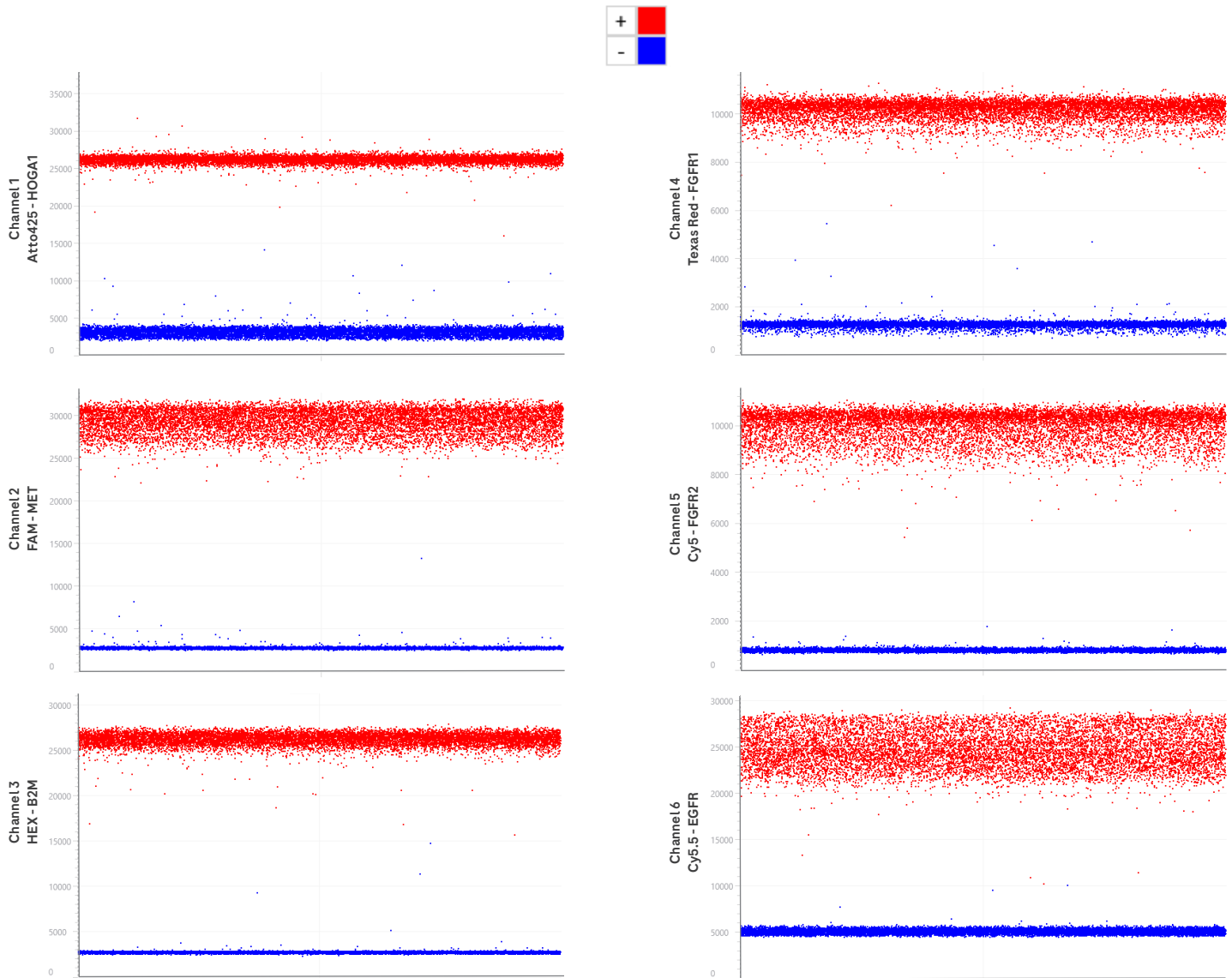


Figure 3. 1D scatter plots of individual channels from the assays shown in Figures 1 and 2. See Figure 4 for additional dyes that are compatible with each channel.

Compatible optical dyes



Figure 4. Dyes compatible with the Digital LightCycler® dPCR System.

Learn more

about how the **Digital LightCycler® dPCR System** supports discriminative multiplexing for up to 6 targets on 6 separate channels at go.roche.com/dpcr or by scanning the QR code.

