Supplementary Information:

Experimental Workflow

Materials

1. **Target gene:** HER2 (Human Epidermal Growth Factor Receptor 2)
2. **Primers and probes:**
    - Forward primer (10 μM)
    - Reverse primer (10 μM)
    - Probe (10 μM)
3. **Reaction components:**
    - Biorad ddPCR supermix (Probe) (2X)
    - Double-distilled water
4. **Lab consumables and equipment:**
    - Filtered pipette tips (China, Shanghai, Sangon Biotech)
    - Droplet generation chips, holders, and sealing pads (self-made)
    - 8-strip tubes (Axygen)
    - Low-adsorption 1.5 mL Eppendorf tubes (Axygen)
    - Mastercycler nexus gradient PCR machine (Eppendorf)
    - QDrop100 droplet generator
    - QX200 droplet reader (Bio-Rad)

Experimental Procedure

1. Reaction System Preparation: Prepare the reaction system as follows (20 μL final volume per reaction):

Component	Final Concentration	Volume (μL)
2X Biorad ddPCR supermix (Probe)	-	10
Forward primer (FAM)	900 nM	0.9
Reverse primer (FAM)	900 nM	0.9
Probe (FAM)	250 nM	0.5
Forward primer (VIC)	900 nM	0.9
Reverse primer (VIC)	900 nM	0.9
Probe (VIC)	250 nM	0.5
Template (HER2)	Suitable amount	2
Double-distilled water	to 20 μL	Adjust volume

2. Reaction Setup

1. Thaw all components stored at 4°C. Briefly centrifuge and vortex to mix.
2. In a clean bench, sequentially add all components in the specified order. Vortex and centrifuge the reaction mixtures before proceeding.

3. Droplet Generation

1. Place 8-strip tubes into the droplet generation chip holder.
2. Add 20 μL of the prepared reaction mix to the small wells of the droplet generation chip in ascending template concentration order.
3. Add 55 μL of self-made droplet generation oil to the large wells of the chip.
4. Seal the chip with the sealing pad and place it into the QDrop100 droplet generator.
5. After droplet generation is complete, retrieve the 8-strip tubes and seal them with caps.

4. PCR Program

Run the PCR using the following cycling parameters:

Step	Temperature (°C)	Time
Initial Denaturation	95	10 min
Denaturation	95	30 sec
Annealing/Extension	60	1 min
Final Extension	60	10 min
Hold	10	-

- Temperature ramp rate: 1°C/s.

5. Droplet Reading and Analysis

1. Load the generated droplets into the Bio-Rad QX200 droplet reader.
2. Follow the Bio-Rad QX200 Droplet Reader User Manual for detailed instructions on data acquisition and analysis.
3. Analyze the fluorescence signal to determine the presence of target DNA.

Table S1:

1. Her2 primers and probes:

Types	**Sequences**
Forwards primer (FAM)	GCAGCATGTCAAGATCACAGATT
Reverse primer (FAM)	CCTCCTTCTGCATGGTATTCTTTCT
Probe (FAM)	FAM-AGTTTGGCCCGCCCAA-MGB-NFQ
Forward primer (VIC)	GTGAAAATTCCTCCTACCC
Reverse primer (VIC)	AAGAGCCAAAGCCCTTAC
Probe (VIC)	VIC-AGTTTGGCCAGCCCAA-MGB-NFQ

2. ALB primers and probes:

Types	**Sequences**
ALB-F	CACACACACACACACACTTAAC
ALB-R	CCCAGAACCTCCAAATTCCA
ALB-P	FAM-TGACAGAGACAAGACCATCATGTGCAA-TAMRA-N

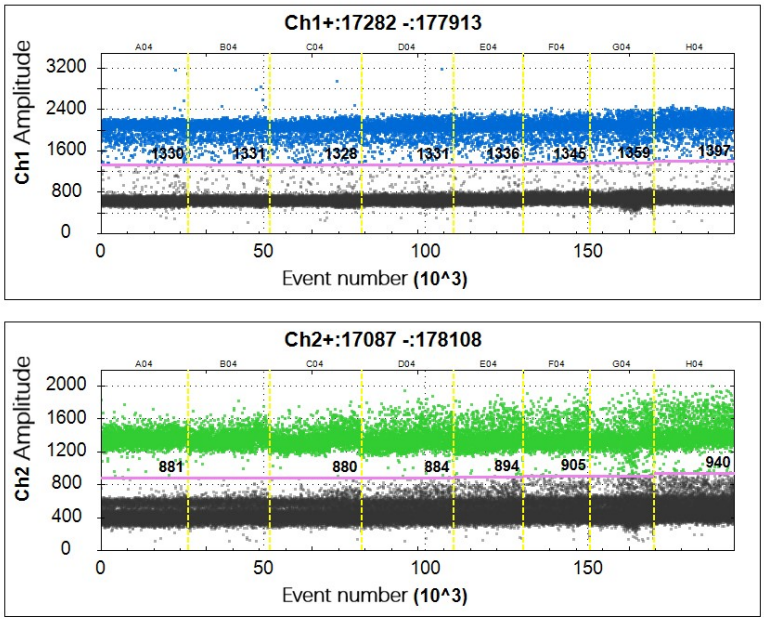

Fig.S1 Droplet 1D fluorescence scatter plot of system reproducibility test. Top refers to FAM channel, down refers to VIC channel. The X axis represents droplet amplitude and Y axis represents droplets fluorescence intensity.

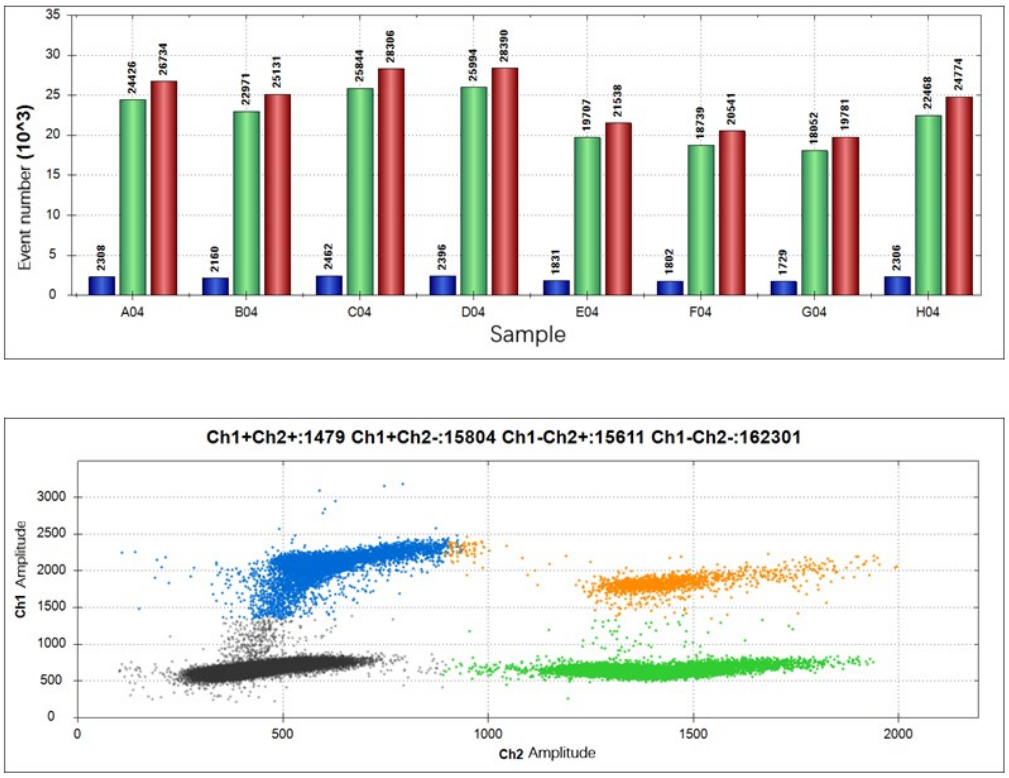

Fig.S 2 Droplet 2D fluorescence scatter plot of system reproducibility test. (Top) Bar chart showing the number of droplets detected in each sample (A04–H04). The different colors represent droplet classification: negative droplets (blue), single-positive droplets (green or red), and double-positive droplets. (Bottom) 2D fluorescence scatter plot illustrating the distribution of droplets based on fluorescence intensity. Ch1 (FAM) intensity is plotted on the y-axis, while Ch2 (VIC) intensity is on the x-axis. Distinct clusters correspond to negative, single-positive (Ch1 or Ch2), and double-positive droplets.

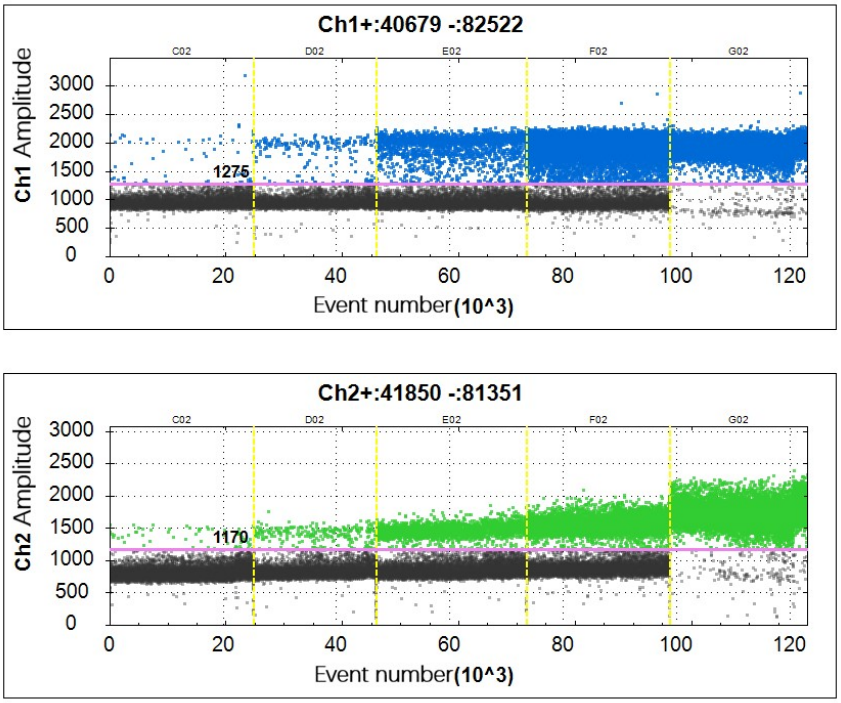

Fig.S3 Droplet 1D fluorescence scatter plot of system Linearity and range test. (Top) Scatter plot for Ch1 (FAM fluorescence channel) showing droplet distribution based on fluorescence intensity. The x-axis represents droplet count (in thousands), and the y-axis indicates Ch1 fluorescence amplitude. Black dots represent negative droplets, while blue dots represent positive droplets containing the target sequence. Yellow vertical lines separate different samples. (Bottom) Scatter plot for Ch2 (HEX fluorescence channel) with similar features. Black dots indicate negative droplets, and green dots indicate positive droplets containing the target sequence.

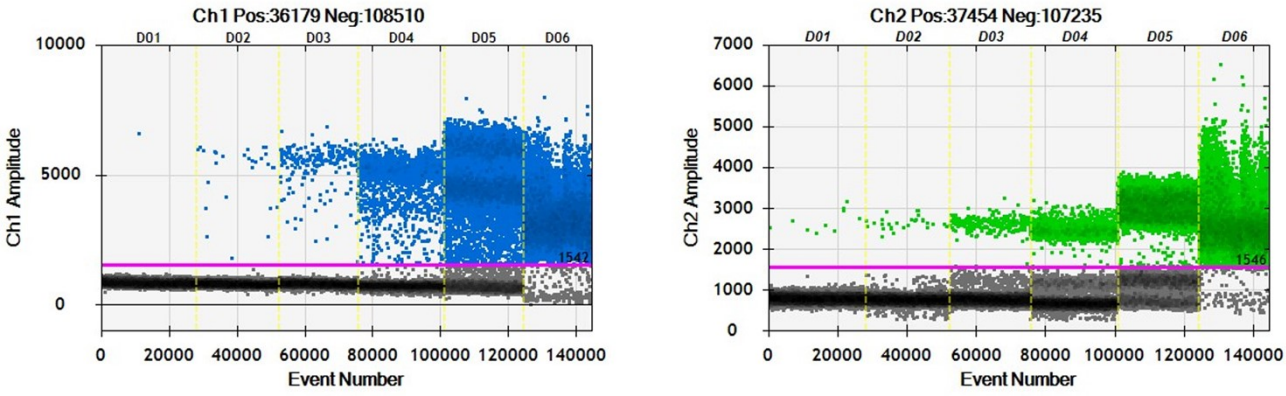

Fig S4. System performing Linearity and range result of Bio-rads system. (Left) Scatter plot for Ch1 (FAM fluorescence channel) showing droplet distribution based on fluorescence intensity. The x-axis represents droplet count (in thousands), and the y-axis indicates Ch1 fluorescence amplitude. Black dots represent negative droplets, while blue dots represent positive droplets containing the target sequence. Yellow vertical lines separate different samples. (Right) Scatter plot for Ch2 (VIC fluorescence channel) with similar features. Black dots indicate negative droplets, and green dots indicate positive droplets containing the target sequence.

	No.1-8	Result n=192 (Unit: um)											
	A1	46.624	46.624	46.624	45.872	46.624	46.624	45.872	45.872	47.376	46.624	46.624	46.624
	A2	45.872	46.624	47.376	46.624	46.624	47.376	46.624	46.624	50.384	46.624	46.624	45.872
	A3	46.624	45.872	45.120	45.872	47.376	45.872	45.872	45.872	50.384	45.872	45.872	45.12
Batch	**A4**	46.624	47.376	45.120	47.376	46.624	46.624	47.376	46.624	47.376	45.120	46.624	46.624
A	**A5**	45.872	47.376	47.376	45.872	44.368	46.624	46.624	47.376	47.376	44.368	45.872	47.376
	A6	46.624	45.872	46.624	46.624	47.376	46.624	46.624	46.624	46.624	46.624	47.376	46.624
	A7	45.872	45.872	46.624	45.872	45.120	45.872	46.624	46.624	46.624	46.624	45.872	45.872
	A8	45.120	46.624	46.624	46.624	46.624	45.120	47.376	47.376	45.872	46.624	46.624	46.624
	B1	44.368	46.624	45.872	46.624	46.624	47.376	45.872	46.624	46.624	46.624	47.376	46.624
	B2	44.368	46.624	45.872	46.624	46.624	47.376	45.872	46.624	46.624	46.624	47.376	45.872
	B3	44.368	45.120	45.120	46.624	46.624	46.624	46.624	46.624	46.624	46.624	45.872	45.872
Batch	**B4**	46.624	46.624	47.376	46.624	47.376	46.624	46.624	46.624	47.376	46.624	48.128	47.376
B	**B5**	45.872	45.120	45.872	45.872	44.368	45.120	45.872	46.624	46.624	46.624	47.376	45.872
	B6	45.872	45.872	45.872	45.872	45.872	45.120	45.872	45.872	45.872	46.624	45.872	46.624
	B7	45.872	45.872	45.872	45.120	45.872	45.872	46.624	46.624	46.624	45.872	45.120	46.624
	B8	45.872	46.624	45.120	46.624	46.624	46.624	45.120	45.872	45.872	45.120	45.120	46.624

Table S2. Measurements result of Batch A/B

Figure S5. Representative Microscopy Images of Microdroplets from Batch A and Batch B

Microscopy images showing water-in-oil droplets generated in Batch A (top panel) and Batch B (bottom panel) across all eight wells (1–8). Droplets exhibit uniform morphology and size distribution, with no observable aggregation or deformation. Scale bar: 34μm (applies to all images).

Table S3. Result of ALB standard measured by Bio-rads systems

Expected	Measured (copies/μL)									CV
1	2.4	2.1	2.2	2.1	1.8	1.5	2.5	2.9	2.3	18.7%
10	11.8	11.6	12.7	12.1	11.2	13.2	11.6	12.4	11.5	6.4%
100	118.5	116.9	111.9	116.1	121.2	109.6	111.2	118.1	113.5	3.7%
1000	1300	1370	1299	1354	1287	1254	1289	1266	1345	3.2%

Table S4. Result of ALB standard measured by QX-100 systems

Expected	Measured (copies/μL)	CV

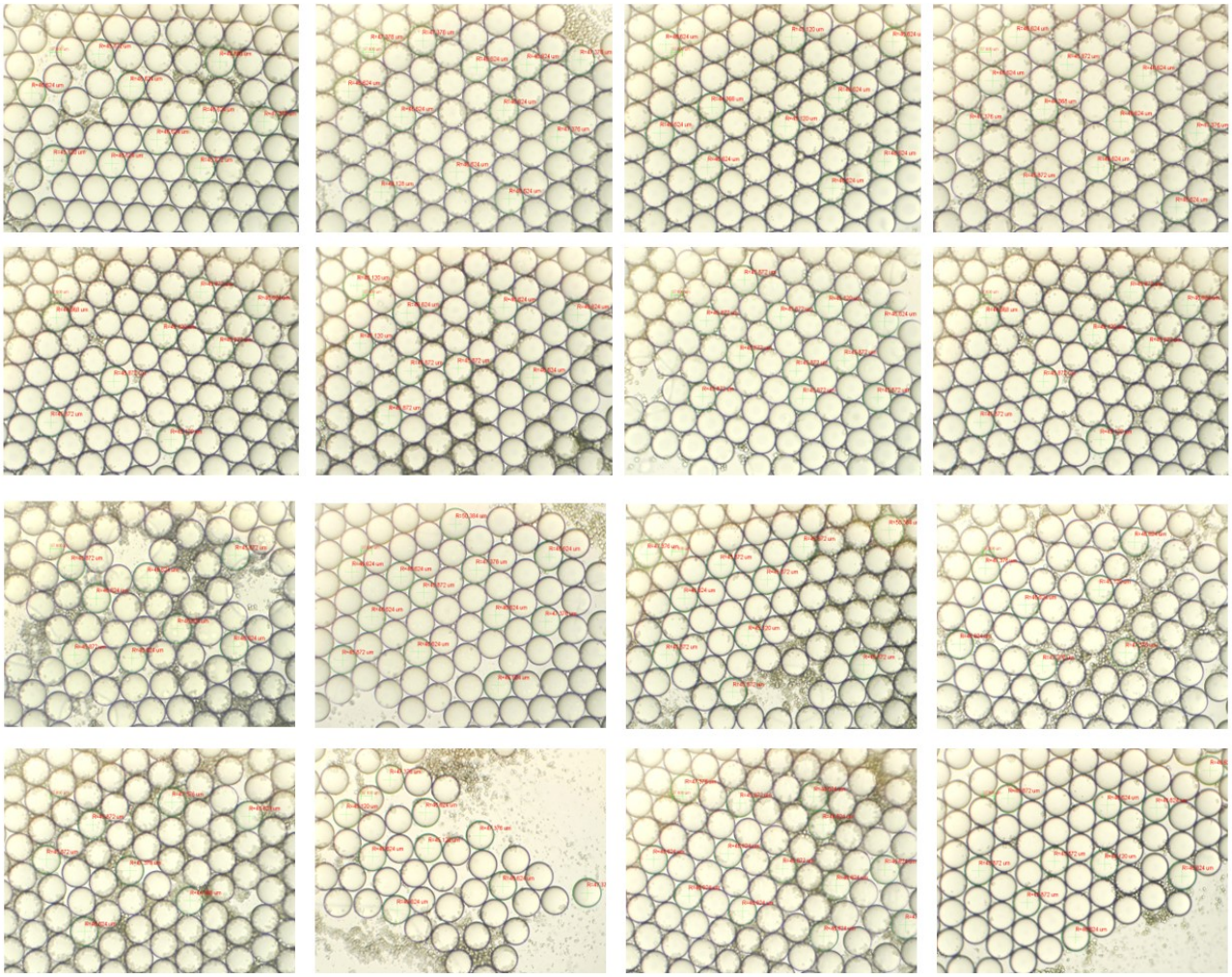

Figure S6. Log-Log Regression of Quantification result of two systems. Left: Bio-rads Right: Qx-100

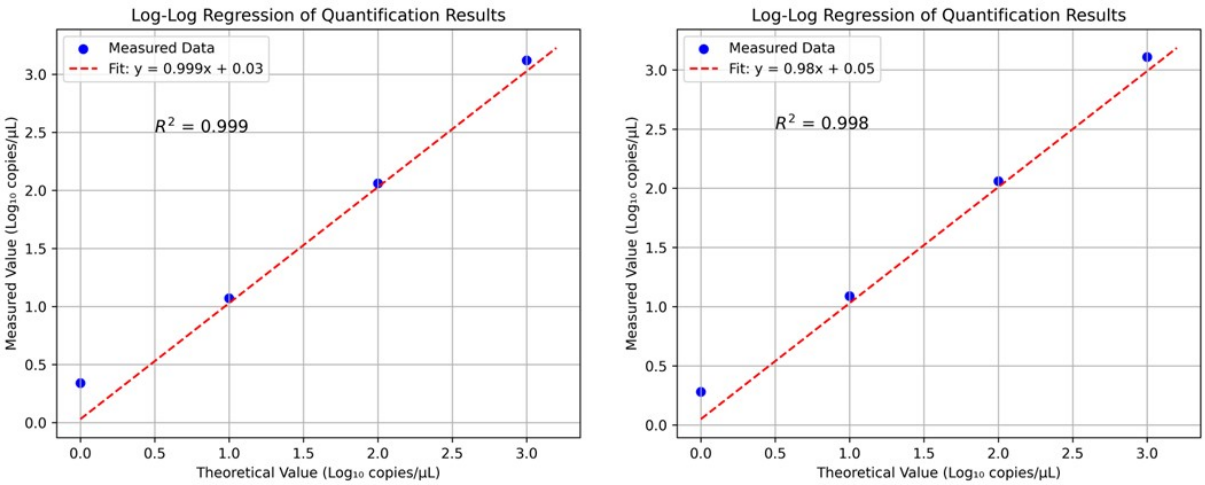

Table S5. Quantitative Accuracy Comparison.

	Recovery Rates	
Concentration (copies/µL)	**Self-Developed System**	**Bio-Rad QX200**
1	191% (±18.8% CV)	219% (±18.7% CV)
10	122% (±13.6% CV)	118% (±6.4% CV)
100	114% (±5.4% CV)	115% (±3.7% CV)
1000	129% (±6.9% CV)	131% (±3.2% CV)