

Materials List for:

Single Droplet Digital Polymerase Chain Reaction for Comprehensive and Simultaneous Detection of Mutations in Hotspot Regions

Charles Decraene^{1,2}, Amanda Bortolini Silveira¹, Marc Michel¹, François-Clément Bidard^{1,3,4}, Jean-Yves Pierga^{1,3,5}, Marc-Henri Stern^{1,6}, Charlotte Proudhon¹

¹Circulating Tumor Biomarkers Laboratory, SiRIC, Translational Research Department, Institut Curie, PSL Research University

²CNRS UMR144, Institut Curie, PSL Research University

³Department of Medical Oncology, Institut Curie, PSL Research University

⁴University Versailles Saint-Quentin-en-Yvelines

⁵University Paris Descartes

⁶Inserm U830, Institut Curie, PSL Research University

Correspondence to: Charlotte Proudhon at Charlotte.Proudhon@curie.fr

URL: <https://www.jove.com/video/58051>

DOI: [doi:10.3791/58051](https://doi.org/10.3791/58051)

Materials

Name	Company	Catalog Number	Comments
K2EDTA tube (color code : lavender)	BD	367863	EDTA tubes are used to obtain a whole blood or EDTA plasma sample
QIAamp Circulating Nucleic Acid Kit (manual protocol)	Qiagen	55114	For isolation of free-circulating DNA from human plasma
QIAasymphony DSP Circulating DNA Kit (automated protocol)	Qiagen	937556	For isolation of free-circulating DNA from human plasma
QIAasymphony SP system	Qiagen	9001297	fully integrated and automated system for cfDNA, DNA or RNA purification
QIAvac 24 Plus	Qiagen	19413	For purification of up to 24 cfDNAs simultaneously
Qubit fluorometer	Invitrogen	Q33226	The Qubit fluorometer is a benchtop fluorometer for the quantitation of DNA
QX100 or QX200 reader	Bio-Rad	186-3003 or 186-4003, respectively	The reader measures fluorescence intensity of each droplet and detects the size and shape as droplets pass the detector
QX100 or QX200 droplet generator	Bio-Rad	186-3002 or 186-4002, respectively	Instrument used for droplet generation
C1000 thermal cycler	Bio-Rad	1851196	Modular thermal cycler platform, includes C1000 Touch thermal cycler chassis, 96-well fast reaction module
Plate sealer	Bio-Rad	181-4000	PX1™ PCR plate sealer
DG8 cartridge holder	Bio-Rad	186-3051	Positions and holds the DG8 cartridge in the instrument for droplet generation
Droplet generator cartridges and gaskets	Bio-Rad	186-4007	Microfluidic DG8 cartridge used to mix sample and oil to generate droplets; DG8 gaskets seal the cartridge to prevent evaporation and apply the pressure required for droplet formation
PCR supermix	Bio-Rad	186-3010	ddPCR supermix for probes (no dUTP)

96-well PCR plates	Eppendorf	951020362	96-well semi-skirted plates
Foil seal	Bio-Rad	181-4040	Pierceable foil heat seal
ddPCR Droplet Reader Oil	Bio-Rad	186-3004	oil used in the read
Droplet Generation Oil for Probes	Bio-Rad	186-3005	oil for droplet generation
DNA loBind Tube 1.5 mL	Eppendorf	0030 108.051	Eppendorf LoBind Tubes maximize sample recovery by significantly reducing sample-to-surface binding
Multiplex I cfDNA Reference Standard Set	HORIZON	HD780	The Multiplex I cfDNA Reference Standards are highly-characterized, biologically-relevant reference materials used to assess the performance of cfDNA assays that detect somatic mutations
QUBIT DSDNA HS ASSAY KIT, 500	Life Technologies	Q32854	The HS assay is highly selective for double-stranded DNA (dsDNA) over RNA and is designed to be accurate for initial sample concentrations from 10 pg/μL to 100 ng/μL
Qubit Assay Tubes	Life Technologies	Q32856	Qubit assay tubes are 500 μL thin-walled polypropylene tubes for use with the Qubit Fluorometer