# *Refine*
PRECISION

KAPA HyperPETE Workflow. Get future ready today.

The KAPA HyperPETE Workflow combines validated products for both KAPA Library Preparation and KAPA Target Enrichment. It provides all accessories and reagents needed for sample preparation in a single-day Primer Extension Target Enrichment (PETE) workflow, from DNA or RNA to the sequencer. The new KAPA HyperPETE Panels provide high capture efficiency and sequencing uniformity with less optimization and higher variant detection confidence. In addition, the workflow will include KAPA Universal Adapters and KAPA UDI Primer Mixes (up to 384*). The KAPA HyperPETE Workflow is fine-tuned for small panel oncology research applications and is supported by NAVIFY® Mutation Caller—an integrated cloud-based analysis solution. NAVIFY® Mutation Caller supports the KAPA HyperPETE Workflow with pipelines for somatic SNV (Single Nucleotide Variant), Indel and CNV (Copy Number Variant) calling, MSI (Micro-Satellite Instability) scoring, RNA fusion detection (known and unknown fusion partners) and germline SNV and Indel calling. The KAPA HyperPETE Workflow also provides the convenience of simplified ordering and support from a single vendor and is automation friendly.

Benefits of the KAPA HyperPETE Workflow

- Roche's renowned design expertise allows variant detection in difficult genomic regions

- Validated with challenging sample types to detect all major mutation classes (SNVs, CNVs, Indels, Fusions including unknown fusion partners and MSI)

- Optimized to deliver accurate molecule counting and low error rates by using the KAPA Universal UMI Adapter

- Outstanding hybrid capture performance in small panels by extensive workflow optimization

- Single-day workflow with short incubations and low hands-on-time

- Convenient all-inclusive workflow with validated products and simplified ordering and support from a single vendor

*KAPA HyperPETE Workflow is validated with the KAPA UDI Primer Mixes 1 – 96 and is theoretically compatible with KAPA UDI Primer Mixes 97 – 384
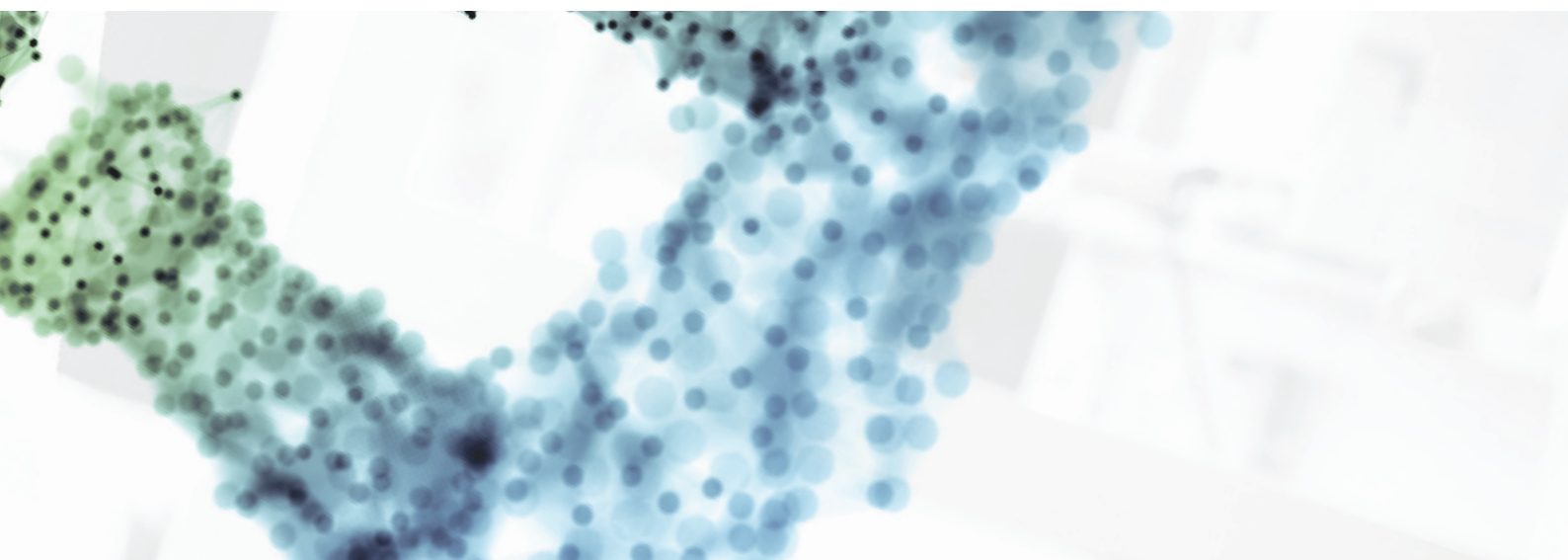
KAPA HyperPETE Catalog Panels. Better by design.

- Better by design, using Roche's renowned content and panel design expertise
- Seamless online generated designs in HyperDesign Tool either with a few clicks or through an expert designer-assisted process
- High uniformity for better sequencing efficiency with less optimization
- Readily available from inventory for fast turn-around-time
- Validated NAVIFY® Mutation Caller secondary analysis solution available

Plasma cfDNA: Variant detection and performance data

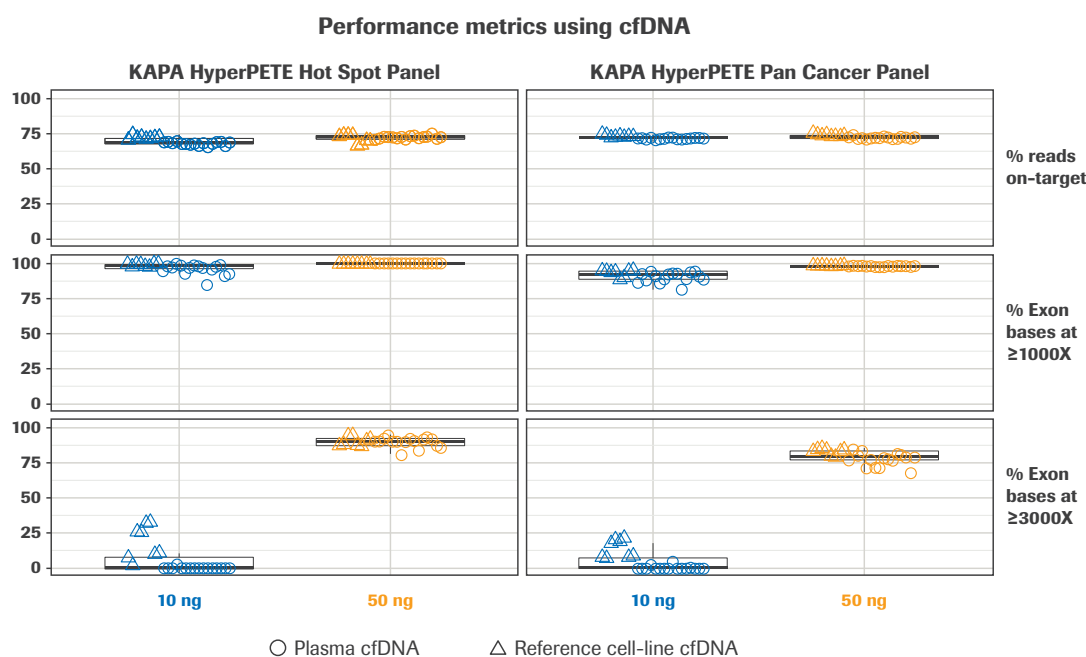### Performance metrics using cfDNA

Figure 1. Performance metrics using plasma and reference cell-line cfDNA. High specificity with deep and broad unique target coverage was demonstrated using the KAPA HyperPETE Workflow for Somatic Plasma cfDNA Preparation across all sample input amounts and types. On-target rate was 65% – 73% for the KAPA HyperPETE Hot Spot Panel and 70% – 73% for the KAPA HyperPETE Pan Cancer Panel. At 50 ng cfDNA input, the percent of panel exon bases covered at ≥1000X of unique depth (PCR duplicates removed) was >99% for the KAPA HyperPETE Hot Spot Panel and 97% – 98% for the KAPA HyperPETE Pan Cancer Panel. The respective percentages at ≥3000X unique depth were 80% – 94% and 67% – 85%. At 10 ng of cfDNA input, the unique depth was concordant with the available unique genome equivalents (~3300 in 10 ng of DNA) and the percentages at ≥1000X unique depth were 83% – 99% and 80% – 94% per panel respectively. Libraries were generated using the KAPA HyperPrep Kit and the KAPA Universal UMI Adapter from either 10 ng or 50 ng of plasma cfDNA or fragmented reference cell-line DNA as input, and individually captured using the KAPA HyperPETE Reagent Kit and the respective panel. Final libraries were sequenced on an Illumina NextSeq™ 550 System with 6M – 14M (median: 8M) high-quality read pairs (2 x 150 bp) allocated per sample for the KAPA HyperPETE Hot Spot Panel, and 55M – 81M (median: 66M) high-quality read pairs allocated per sample for the KAPA HyperPETE Pan Cancer Panel. Two (2) Seraseq® ctDNA Mutation Mix samples in duplicates and sixteen (16) healthy donor plasma cfDNA samples were used to assess the performance. Data was analyzed using the NAVIFY® Mutation Caller.

Table 1. Variant detection performance using reference cell-line cfDNA

Panel	Input (ng)	Allele frequency	Expected variants	Detected variants	True positive rate
KAPA HyperPETE Hot Spot Panel	10	1.0%	56	56	100%
	50			56	100%
	10	0.5%	50	47*	94%
	50			50	100%
KAPA HyperPETE Pan Cancer Panel	10	1.0%	70	70	100%
	50			70	100%
	10	0.5%	64	62*	96.9%
	50			63*	98.4%

High true positive detection rates were demonstrated for short variants (Single Nucleotide Variants—SNVs and Indels) at low variant allele expected frequencies across all reference cell line samples (two Seraseq® ctDNA Mutation Mix samples in duplicates). From both input amounts (10 ng and 50 ng) 100% of short variants (SNVs and Indels) were detected at 1% allele frequency using either of the two KAPA HyperPETE Panels. At 0.5% allele frequency from 10 ng input, the true positive detection rate was 94% and 96.9% using the KAPA HyperPETE Hot Spot Panel and the KAPA HyperPETE Pan Cancer Panel, respectively. At 0.5% allele frequency from 50 ng input, the true positive detection rate was 100% and 98.4% using the KAPA HyperPETE Hot Spot Panel and the KAPA HyperPETE Pan Cancer Panel, respectively.

Tissue RNA (FFPET): Fusion detection and performance data

Performance using FFPE RNA with the KAPA HyperPETE LC Fusion Panel

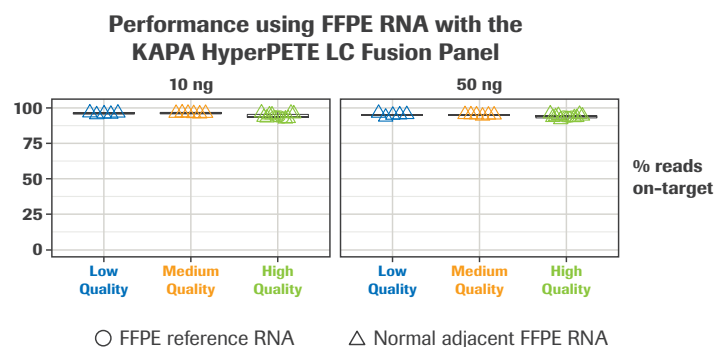○ FFPE reference RNA   △ Normal adjacent FFPE RNA

Figure 2. Performance using tissue RNA (FFPET). High specificity was demonstrated by the high percent of reads on-target when starting from 10 ng or 50 ng of various quality (low, medium, high) input FFPE RNA. On-target rate was 92% to 97% (includes housekeeping and fusion targets) with good performance across all sample input amounts, qualities, and types. The on-target rate was calculated following rRNA read removal (0.8% to 11% of all reads). Two (2) cell-line samples (in duplicates) and fourteen (14) normal adjacent FFPET samples were used to assess performance. RNA was extracted with the Roche High Pure FFPET RNA Isolation Kit and quality was determined with the DV200 score using the Agilent RNA 6000 Pico Assay on the Bioanalyzer. The KAPA HyperPETE Workflow for Tissue RNA Fusion Transcript Preparation using the KAPA HyperPETE LC Fusion Panel was followed. Libraries were generated using the KAPA RNA HyperPrep kit in combination with the KAPA Universal UMI Adapter and either 10 ng or 50 ng of RNA while adjusting PCR cycles based on the input amount and DV200 score. Libraries were captured using the KAPA HyperPETE Reagent Kit and sequenced on an Illumina NextSeq™ 550 System. Total read pairs (2 x 150 bp) per sample ranged from 3.6M to 17M and data was analyzed using NAVIFY® Mutation Caller to assess enrichment and variant detection performance.

Table 2. Fusion detection performance using tissue RNA (FFPET)

Variant type	RNA input amount	Total replicates	Expected variants	True positive rate
Fusion	10 ng	6	62	100%
Fusion	50 ng	6	62	100%

All fusions (100%) were detected in the reference cell line samples at both 10 ng and 50 ng RNA input amounts. Two (2) Seraseq® RNA Fusion FFPE samples and 1 Horizon Discovery RNA Fusion FFPE sample, each run in duplicate, were used to assess fusion detection performance. The EGFR-SEPT14 variant in Seraseq® Fusion RNA Mix v4 was manually curated as the fusion caller in NAVIFY® Mutation Caller identified an *EGFR* partner that has a homologous sequence to *SEPT14*. Comparable variant detection results were achieved when down-sampling to 1M read pairs (2 x 150 bp, data not shown).

KAPA HyperPETE Custom Design Expertise

- Quickly and efficiently cover custom regions with high-performing KAPA HyperPETE Custom Panels
  - Rely on Roche's proven design and primer selection expertise to improve your desired target coverage and maximize the data return from your research. Use the HyperDesign Tool to prepare your custom design in four (4) simple steps, include an optimized MSI target set into your design or consult with Roche's expert designer team for your special design needs.

- Take fewer steps to optimal performance and sequencing efficiency
  - Experience high performance from your very first panel design iteration as a result of Roche's design expertise and extensive KAPA HyperPETE Workflow optimization

- Achieve confident variant detection for all major mutation classes
  - Detect SNVs, Indels, CNVs, Fusions, and MSI in your regions of interest, using an optimized workflow with integrated secondary analysis by the NAVIFY® Mutation Caller

Tissue DNA (FFPET): Variant detection and performance data

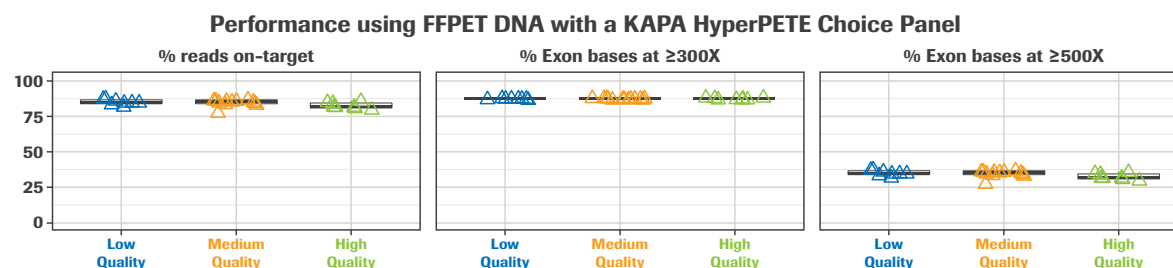**Performance using FFPET DNA with a KAPA HyperPETE Choice Panel**

Figure 3. Performance using tissue DNA (FFPET). High specificity with deep and broad target coverage was demonstrated using a KAPA HyperPETE Choice Panel (capture target of 88 Kb plus 25 Kb of the Roche preset MSI targets). Percent of reads on-target ranged from 77.1% to 87.7% (median 84.4%), percent of bases covered at ≥300X ranged from 97.2% to 98.7% (median 98.4%), and percent of bases covered at ≥500X ranged from 94.4% to 98.3% (median 97.4%). Eight (8) FFPET DNA samples and eight (8) control FFPET DNA samples of varying quality were tested in duplicates by following the KAPA HyperPETE Workflow for Somatic Tissue DNA Preparation. High-quality DNA samples had a normalized Q score ≥0.4 with input range of 20 ng to 34.4 ng, medium quality ≥0.22 with input range of 37 ng to 55.5 ng, and low quality ≥0.09 with input range of 62.6 ng to 121.1 ng. Final libraries were sequenced on an Illumina NextSeq™ 550 System. Total read pairs (2 x 150 bp) per sample ranged from 12.5M to 20.4M and data was analyzed using NAVIFY® Mutation Caller to assess enrichment and variant detection performance.

Table 3 (a, b, c, d). Highly confident detection of SNVs, Indels, CNV, and MSI using Horizon Discovery FFPET control and CrownBio FFPET xenograft samples

Table 3a. Performance in SNV and Indel detection

Sample	Input (ng)	Expected allele frequency	Total replicates	Expected variants	False negatives	True positive rate
HD200	20	5%	4	24 SNVs	0	100%
HD200	20	5%	4	4 Indels	0	100%
HD789	20	5%	4	8 Indels	0	100%

Table 3b. Performance in CNV detection

Sample	Input (ng)	Gene	Total replicates	Expected copies	False negatives	True positive rate
HD789	20	MET	4	4.5	0	100%

Table 3c. Performance in MSI detection (true positive rate)

Sample (xenografts)	Input (ng)	Expected status	Total replicates	Reported status (≥40% = MSI)	True positive rate
DU145	62.6 – 343.3	MSI	2	MSI: 83.1% – 84.3%	100%
SW48	41.3 – 45.7	MSI	2	MSI: 97.1% – 98.3%	100%

Table 3d. Performance in MSI detection (false positive rate)

Sample (xenografts)	Input (ng)	Expected status	Total replicates	Reported status (<40% = MSS)	False positive rate
BT474	40.3 – 51.7	MSS (Microsatellite stable)	2	MSS: 23.4% – 29.2%	0%
MDA-MB-453	72.5	MSS (Microsatellite stable)	1	MSS: 28.5%	0%

Performance was demonstrated using a KAPA HyperPETE Choice Panel (capture target of 88 Kb plus 25 Kb of the Roche preset MSI targets). True positive detection rates were 100% for SNVs, or Indels (Table 3a), CNVs (Table 3b) and MSI (Table 3c), without any false negatives. False positive rate was 0% for microsatellite stable samples (Table 3d). Final libraries were sequenced on an Illumina NextSeq™ 550 System. Total read pairs (2x150 bp) per sample ranged from 12.5M to 20.4M and data was analyzed using NAVIFY® Mutation Caller to assess the variant detection performance.

Superior performance and coverage uniformity

- Achieve more uniform coverage compared to anchored multiplex PCR (Figure 4)

- Avoid over- or under-representation of PCR-based target regions; KAPA HyperPETE technology uses primers to capture the regions of interest, not to amplify them

- Streamline bioinformatics pipelines and increase your sequencing efficiency by eliminating the need to remove primer binding site sequences from your reads
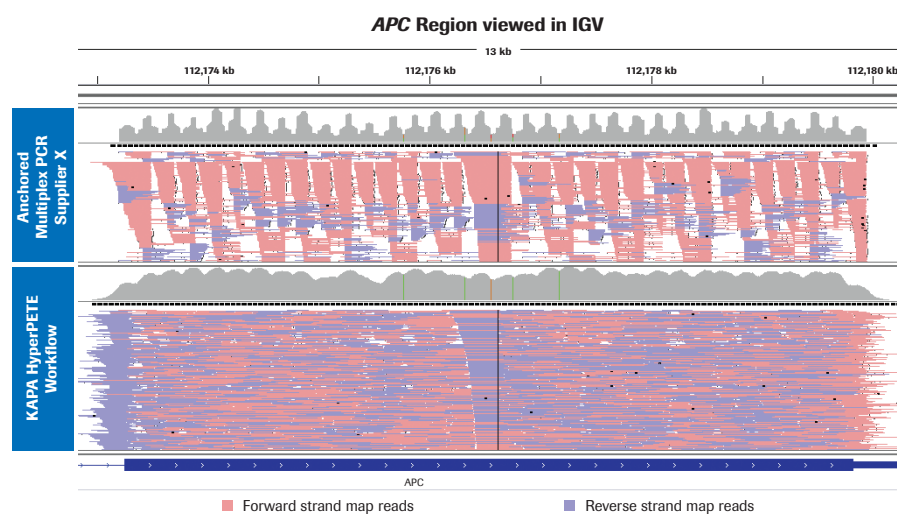
APC Region viewed in IGV

Forward strand map reads   Reverse strand map reads

Figure 4. KAPA HyperPETE achieves greater uniformity across the APC region compared to anchored multiplex PCR. In this IGV (Integrative Genomics Viewer) image, white space indicates a lack of coverage. In the anchored multiplex PCR workflow, the amplification primers (which contain adapters) are fixed on one end and flexible on the other end, reducing coverage uniformity. In the KAPA HyperPETE Workflow, the primer extension and subsequent steps are used to capture full-length library molecules, which are then sequenced; this leads to improved coverage uniformity.

Ordering Information for Nucleic acid purification, QC, Polishing, and Reagent Kits

Roche cat. no.	Description	Pack size
09189823001	KAPA NGS DNA Extraction Kit, 24 rxn	24 reactions
09190023001	KAPA NGS DNA Extraction Kit, 96 rxn	96 reactions
09217193001	KAPA NGS FFPE DNA QC Kit, 24 rxn	24 reactions
09217207001	KAPA NGS FFPE DNA QC Kit, 96 rxn	96 reactions*
09217215001	KAPA NGS FFPE DNA Polishing Kit, 24 rxn	24 reactions
09217223001	KAPA NGS FFPE DNA Polishing Kit, 96 rxn	96 reactions
09211624001	KAPA HyperPETE Reagent Kit, 24 rxn	24 reactions
09211683001	KAPA HyperPETE Reagent Kit, 96 rxn	96 reactions

*Virtual kit

Ordering Information for recommended on-market KAPA Library Preparation Kits

Roche cat. no.	Description	Pack size
07962312001	KAPA HyperPrep Kit, 8 rxn[1]	8 reactions
07962347001	KAPA HyperPrep Kit, 24 rxn[1]	24 reactions
07962363001	KAPA HyperPrep Kit, 96 rxn[1]	96 reactions
07962380001	KAPA HyperPlus Kit, 8 rxn[2]	8 reactions
07962401001	KAPA HyperPlus Kit, 24 rxn[2]	24 reactions
07962428001	KAPA HyperPlus Kit, 96 rxn[2]	96 reactions
08098093702	KAPA RNA HyperPrep Kit, 24 rxn[3]	24 reactions
08098107702	KAPA RNA HyperPrep Kit, 96 rxn[3]	96 reactions

[1]for Plasma cell-free DNA and High-quality DNA
[2]for FFPE Tissue DNA and High-quality DNA
[3]for FFPE Tissue RNA

Ordering Information for Accessory and Reagent Kits

Roche cat. no.	Description	Pack size
09063781001	KAPA Universal Adapter, 15 µM 960 µL	96 samples
09063790001	KAPA Universal Adapter, 15 µM 4 x 960 µL	384 samples*
09329862001	KAPA Universal UMI Adapter, 960 µL	96 samples
09329889001	KAPA Universal UMI Adapter, 4 x 960 µL	384 samples*
09134336001	KAPA UDI Primer Mixes, 1 – 96, 96 rxn	96 samples
09329838001	KAPA UDI Primer Mixes, 97 – 192, 96 rxn	96 samples**
09329846001	KAPA UDI Primer Mixes, 193 – 288, 96 rxn	96 samples**
09329854001	KAPA UDI Primer Mixes, 289 – 384, 96 rxn	96 samples**
09075780001	KAPA HyperCapture Bead Kit, 24 rxn	24 reactions
09075798001	KAPA HyperCapture Bead Kit, 96 rxn	96 reactions
09075909001	KAPA HyperCapture Bead Kit, 384 rxn	384 reactions*
08963835001	KAPA HyperPure Beads	5 mL
08963843001	KAPA HyperPure Beads	30 mL
08963851001	KAPA HyperPure Beads	60 mL
08963878001	KAPA HyperPure Beads	4 x 60 mL
08963860001	KAPA HyperPure Beads	450 mL

*Virtual kit
**KAPA HyperPETE Workflow is validated with the KAPA UDI Primer Mixes 1 – 96 and is theoretically compatible with KAPA UDI Primer Mixes 97 – 384

Ordering Information for KAPA HyperPETE Catalog Panels

Roche cat. no.	Description	Pack size
09329161001	KAPA HyperPETE Pan Cancer Panel	24 reactions
09329196001	KAPA HyperPETE Pan Cancer Panel	96 reactions
09329226001	KAPA HyperPETE Pan Cancer Panel	384 reactions
09329234001	KAPA HyperPETE Hot Spot Panel	24 reactions
09329277001	KAPA HyperPETE Hot Spot Panel	96 reactions
09329307001	KAPA HyperPETE Hot Spot Panel	384 reactions
09329315001	KAPA HyperPETE Hereditary Oncology Panel	24 reactions
09329340001	KAPA HyperPETE Hereditary Oncology Panel	96 reactions
09329374001	KAPA HyperPETE Hereditary Oncology Panel	384 reactions
09329382001	KAPA HyperPETE Newborn Screening Panel[†]	24 reactions
09329439001	KAPA HyperPETE Newborn Screening Panel[†]	96 reactions
09329463001	KAPA HyperPETE Newborn Screening Panel[†]	384 reactions
09329471001	KAPA HyperPETE LC Fusion Panel	24 reactions
09329501001	KAPA HyperPETE LC Fusion Panel	96 reactions
09329536001	KAPA HyperPETE LC Fusion Panel	384 reactions

[†]Not available for sale in the United States. Contact the local Roche affiliate for availability in other regions.

Ordering Information for KAPA HyperPETE Choice Panels

Roche cat. no.	Description	Pack size
Varies	KAPA HyperPETE Choice 75Kb	96, 384, 1536, or 10000 reactions
Varies	KAPA HyperPETE Choice 150Kb	96, 384, 1536, or 10000 reactions
Varies	KAPA HyperPETE Choice 250Kb	96, 384, 1536, or 10000 reactions
Varies	KAPA HyperPETE Choice RNA 50Kb	96, 384, or 1536 reactions

Ordering Information for KAPA HyperPETE Explore Panels

Roche cat. no.	Description	Pack size
Varies	KAPA HyperPETE Explore 75Kb	96, 384, 1536, or 10000 reactions
Varies	KAPA HyperPETE Explore 150Kb	96, 384, 1536, or 10000 reactions
Varies	KAPA HyperPETE Explore 250Kb	96, 384, 1536, or 10000 reactions
Varies	KAPA HyperPETE Explore RNA 50Kb	96, 384, or 1536 reactions

Learn more at
sequencing.roche.com

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