

TruSeq™ Custom Amplicon Dx – FFPE QC Kit

FOR IN VITRO DIAGNOSTIC USE

Catalog # 20006259: 1-4 uses, up to 48 Samples

Intended Use

The Illumina TruSeq Custom Amplicon Dx – FFPE QC Kit is a set of reagents used to determine the amplification potential of genomic DNA (gDNA) extracted from formalin-fixed, paraffin-embedded (FFPE) samples.

Principles of Procedure

The Illumina TruSeq Custom Amplicon Dx – FFPE QC Kit is intended for evaluating the quality of prospective DNA samples from FFPE tissue to determine if they are viable for use with the TruSeq Custom Amplicon Kit Dx or other library preparation kits. The kit uses a quantitative real-time PCR (qPCR) assay that can be carried out using standard instrumentation. The qPCR determines amplification potential of DNA extracted from FFPE samples.

FFPE gDNA input requirements for library preparation are based on the delta quantitative cycle (dCq) obtained from the kit. The dCq is the difference between the cycle at which a sample and a control each pass a threshold. The reagents provided in the TruSeq Custom Amplicon Dx – FFPE QC Kit specifically amplify repeated regions throughout the genome. The quantity of libraries depends on the quantity of amplifiable gDNA extracted from FFPE samples. The higher the dCq of the samples, the lower the quantity of amplifiable gDNA, and the higher the amount of input DNA required for library preparation.

Limitations of the Procedure

- 1 For *in vitro* diagnostic use.

Product Components

The Illumina TruSeq Custom Amplicon Dx – FFPE QC Kit consists of the following:

- TruSeq Custom Amplicon Dx – FFPE QC Kit (Catalog # 20006259)

Reagents

Reagents Provided

The Illumina TruSeq Custom Amplicon Dx – FFPE QC Kit has been configured to process 48 samples. The kit supports four uses with 12 samples per use.

See the following tables for a complete list of reagents provided in this kit.

TruSeq Custom Amplicon Dx – FFPE QC Kit

Table 1 Box 1 Pre-Amp Reagents

Component	Quantity	Fill Volume	Active Ingredients	Storage
qPCR Master Mix	2 tubes	1 ml	Buffered aqueous solution containing salts, dNTPs, DNA polymerase, passive reference, and green fluorescent dye (SYBR)	-25°C to -15°C
QC Primers	4 tubes	75 µl	Buffered aqueous solution containing oligonucleotides (primers) for DNA sample qualification	-25°C to -15°C

Reagents Required, Not Provided

- 1X TE Buffer (10 mM Tris, 1 mM EDTA, pH 8.0)
- RNase/DNase-free water

Storage and Handling

- 1 Room temperature is defined as 15°C to 30°C.
- 2 The following reagents are shipped frozen and are stable when stored at -25°C to -15°C until the specified expiration date.
 - ▶ qPCR Master Mix
 - ▶ QC Primers

The reagents are stable for a maximum of six freeze-thaw cycles that occur before the specified expiration date.
- 3 Changes in the physical appearance of the reagents provided can indicate deterioration of the materials. If changes in the physical appearance occur (for example, obvious changes in reagent color or cloudiness apparent with microbial contamination), do not use the reagents.

Equipment and Materials

Equipment and Materials Required, Not Provided

Pre-Amp Equipment and Materials

- 1 **Tabletop Centrifuge**—One tabletop centrifuge placed either in the pre- or post-amp laboratory area. The centrifuge must meet the following specifications.
 - ▶ Can maintain 20°C
 - ▶ Fits a 96- or 384-well plate
 - ▶ Accepts 5 ml tubes
 - ▶ Attains speeds of 280 to 2400 × g
- 2 **Precision Pipettes**—One set of precision pipettes is required. The use of precision pipettes ensures accurate reagent and sample delivery. Single channel and/or multichannel pipettes can be used if they are calibrated regularly and are accurate within 5% of stated volume.
- 3 **Consumables**—The following consumables are required.
 - ▶ 1.5 ml or 2 ml tubes
 - ▶ 8-tube strips and caps
 - ▶ 96- or 384-well PCR plates compatible with qPCR instrument, 0.2 ml, polypropylene, or equivalent
 - ▶ Solution basin, PVC, DNase, RNase-free (trough)
 - ▶ Seal compatible with qPCR instrument
 - ▶ Aerosol resistant pipette tips
- 4 **Microcentrifuge**
- 5 **Vortexer**

- 6 **Quality Control DNA**—High molecular weight, double-stranded human DNA available from commercial vendors or isolated from human blood.

Post-Amp Equipment and Materials

- 1 **qPCR thermal cycler**—One quantitative PCR instrument is required. The instrument must have a heated lid and have the ability to detect the SYBR dye (FAM channel; excitation filter of ~490 nm and emission filter of ~520 nm).

Warnings and Precautions



CAUTION

Federal law restricts this device to sale by or on the order of a physician or other practitioner licensed by the law of the State in which he/she practices, to use or order the use of the device.



WARNING

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, see the SDS at support.illumina.com/sds.html.

- 1 Handle all blood specimens as if they are known to be infectious for Human Immunodeficiency Virus (HIV), Human hepatitis B virus (HBV), and other blood borne pathogens (universal precautions).
- 2 Failure to follow the procedures as outlined may result in erroneous results or significant reduction in sample quality.
- 3 Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink, or smoke in designated work areas. Wear disposable gloves and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.
- 4 Do not use any kit components beyond their stated expiration date on the kit carton label. Do not interchange kit components from different kit lots. Note that kit lots are identified on the kit carton label.
- 5 Store the kit components at the specified temperature in designated pre-amplification and post-amplification areas.
- 6 Avoid repeated freeze-thaw cycles of the reagents. Refer to *Procedural Notes on page 4* for the number of uses of the kit.
- 7 To prevent sample or reagent degradation, make sure that all sodium hypochlorite vapors have fully dissipated before starting the protocol.
- 8 Proper laboratory practices and good laboratory hygiene are required to prevent PCR products from contaminating reagents, instrumentation, and genomic DNA samples. PCR contamination may cause inaccurate and unreliable results.
- 9 To prevent contamination, make sure that pre-amplification and post-amplification areas have dedicated equipment (for example, pipettes, pipette tips, vortexer, and centrifuge).
- 10 Avoid cross-contamination. Use fresh pipette tips between samples and between dispensing reagents. Mix samples with a pipette and centrifuge the plate when indicated. Do not vortex the plates. Using aerosol-resistant tips reduces the risk of amplicon carry-over and sample-to-sample cross-contamination.
- 11 Quantification methods depend on accurate pipetting methods. Do not use pipettes at the extremes of volume specifications. Make sure that pipettes are calibrated.

Acronyms

Table 2 Illumina TruSeq Custom Amplicon Dx – FFPE QC Kit
Acronyms

Acronym	Definition
NTC	No Template Control
qPCR	Quantitative Polymerase Chain Reaction

Specimen Collection, Transport, and Storage

The following conditions should be met when handling tumor tissue and DNA extracted from the tissue.

- 1 Tumor tissue should be formalin-fixed and paraffin-embedded.
- 2 Extracted gDNA should be kept between 2°C and 8°C for a maximum of 28 days or stored frozen between -15°C to -25°C for a maximum of 161 days.
- 3 Frozen gDNA samples are stable for two freeze-thaw cycles.

DNA Extraction

Illumina recommends column-based DNA extraction kits, using double the amount of Proteinase K, overnight Proteinase K incubations with agitation, and final elutions in at least a 30 µl volume. Bead-based extraction methods and methods using only lysis of crude cell extracts are not recommended for use with these reagents.



NOTE

No adverse effect on kit performance was observed with FFPE tissue when trace amounts of Deparaffinization Solution, paraffin wax, xylene, ethanol, Proteinase K, wash solutions, hemoglobin, or necrotic tissue was present.

Procedural Notes

- 1 The kit can be used up to four times if fewer than 96 samples are screened.
- 2 Illumina requires that a negative control (NTC or No Template Control) is included in every use.
- 3 Qualify the DNA using the Illumina TruSeq Custom Amplicon Dx – FFPE QC Kit, as described in *Instructions for Use*. Library yield and sequencing performance are dependent on sample quality as measured by the TruSeq Custom Amplicon Dx – FFPE QC Kit.

Instructions for Use

Preparation

- 1 Bring the Quality Control DNA, QC Primers, qPCR Master Mix, and gDNA to room temperature.
- 2 Vortex the QC Primers vigorously and briefly centrifuge the tubes to collect liquid.
- 3 Invert the Control DNA, gDNA, and qPCR Master Mix 10 times and briefly centrifuge the tubes to collect liquid.
- 4 Place all tubes on ice and shield the qPCR Master Mix from ambient light.
- 5 Determine the plate layout of the qPCR reaction (use [Figure 1 on page 5](#) as a guide).

Procedure

- 1 Prepare quality control DNA by choosing one of the following options:
 - ▶ **[Option 1] Commercially Available gDNA** — Dilute the DNA based on the concentration supplied by the vendor. Prepare at least 50 µl of Quality Control DNA at a concentration of 0.25 ng/µl using 1X TE Buffer.

- ▶ **[Option 2] Extracted gDNA** — Determine the concentration with a spectrophotometer and 1X TE Buffer as the blank. Measure the gDNA sample in triplicate. The % CV must be less than or equal to 20%. Repeat sample readings if % CV is greater than 20%. Prepare at least 50 µl of Quality Control DNA freshly diluted to 0.25 ng/µl using 1X TE Buffer.
- 2 Determine the plate layout of the qPCR reaction (**Figure 1**). Test the Control DNA, NTC, and each sample gDNA in triplicate. To calculate the number of wells, perform the following step:
 - ▶ Number of wells total = 3 × [1 (Control DNA) + 1 (NTC) + # of gDNA samples]
- 3 In a PCR 8-tube strip, combine 148.5 µl of 1X TE Buffer and 1.5 µl of Sample gDNA to make a 100-fold dilution.
- 4 Using a P200 multichannel pipette set to 100 µl, pipette up and down 10 times to mix the dilutions.
- 5 Transfer 30 µl of 0.25 ng/µl control DNA dilution to an unused well in the PCR 8-tube strip.

Figure 1 Suggested Plate Layout for qPCR

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1	Sample 1	Sample 1	Sample 9	Sample 9	Sample 9						
B	Sample 2	Sample 2	Sample 2	Sample 10	Sample 10	Sample 10						
C	Sample 3	Sample 3	Sample 3	Sample 11	Sample 11	Sample 11						
D	Sample 4	Sample 4	Sample 4	Sample 12	Sample 12	Sample 12						
E	Sample 5	Sample 5	Sample 5	Sample 13	Sample 13	Sample 13						
F	Sample 6	Sample 6	Sample 6	Sample 14	Sample 14	Sample 14						
G	Sample 7	Sample 7	Sample 7	Control DNA	Control DNA	Control DNA						
H	Sample 8	Sample 8	Sample 8	NTC	NTC	NTC						

- 6 Add 150 µl of 1X TE Buffer to another unused well for use as the NTC.
- 7 Cap the 8-tube strips and briefly centrifuge to collect the liquid.
- 8 Prepare a sufficient quantity of qPCR reaction mix for a 384-well or 96-well plate format based on the number of reactions determined in step 2. **Table 3** lists the volumes of each component for a single reaction. Include extra volume for pipetting error.

Table 3 qPCR Reaction Mix

Reaction Mix Component	384-Well Vol (µl)	96-Well Vol (µl)
qPCR Master Mix	5	10
QC Primers	0.8	1.6
Water	2.2	4.4
Vol reaction mix per well	8	16
Sample	2	4
Total reaction volume per well	10	20

- 9 Mix the reaction mix gently but thoroughly. Briefly centrifuge to collect the liquid. Place the reaction mix on ice and protect it from light until use.
- 10 Aliquot reaction mix into a trough or 8-tube strip to aid in dispensing with multichannel pipette.
- 11 Add 8 µl (384-well format) or 16 µl (96-well format) of the qPCR Reaction Mix to each sample well of the qPCR plate.

**CAUTION**

Make sure to pipette accurately; small variations will affect the assay.

- 12 Add 2 μl (384-well format) or 4 μl (96-well format) of 0.25 ng/ μl dilution of control DNA, the gDNA sample dilutions, or 1X TE Buffer to each well of the plate (see [Figure 1](#) for suggestions).

**CAUTION**

Make sure to pipette accurately; small variations will affect the assay.

- 13 Using a P20 multichannel pipette set at half the total reaction volume (5 μl for 384-well plate or 10 μl for 96-well plate), slowly pipette up and down three times to mix.
- 14 Seal the plate with an optically clear seal, taking care to avoid cross-contamination and to avoid smudging the surface of the seal.
- 15 Centrifuge the plate at 1000 g at 20°C for 1 minute.
- 16 Make sure that the seal and plate are free of any liquid or dust, place the plate in the qPCR instrument in the correct orientation, and then close the lid and run the following qPCR thermal profile (with a heated lid):
 - ▶ 50°C for 2 minutes
 - ▶ 95°C for 10 minutes
 - ▶ 40 cycles of:
 - ▶ 95°C for 30 seconds
 - ▶ 57°C for 30 seconds
 - ▶ 72°C for 30 seconds
- 17 Confirm that the instrument captures images after the 72°C step in step 16.
- 18 Average the Cq value of triplicate reactions of the Control DNA, NTC, and each sample. Treat outliers as specified in [Quality Control Procedures on page 6](#).
- 19 Subtract the average Cq of the Control DNA from the average Cq of each sample (Sample Average Cq minus Control DNA Average Cq) to yield the dCq values for each sample. Record the dCq values, any replicates that were excluded, and the sample dilution factors. For samples with $dCq \leq -1.5$, dilute sample 16-fold, and repeat dCq measurement until value is > -1.5 . For library preparation using the TSCA Kit Dx, follow sample dilution instructions for the applicable group:
 - ▶ $-1.5 < dCq \leq -0.5$, dilute sample eight-fold
 - ▶ $-0.5 < dCq \leq 0.5$, dilute sample four-fold
 - ▶ $0.5 < dCq \leq 1.5$, dilute sample two-fold
 - ▶ $1.5 < dCq \leq 4$, use undiluted sample
 - ▶ $dCq > 4$, do not use sample

SAFE STOPPING POINT

The dCq values are valid for 28 days if the DNA samples are stored at 2°C to 8°C; they are valid for 161 days if the DNA samples are stored at -25°C to -15°C.

Quality Control Procedures

- A Quality Control DNA and a negative (no-template) control are included in every qualification qPCR run. The Quality Control DNA template is used to normalize the qPCR data.
- After the final step, the qPCR instrument analyzes the quantified samples. If amplification of the NTC occurs within 10 cycles of the Quality Control DNA amplification, contamination of samples is likely and testing must be repeated.
- Make sure that the Quality Control DNA produces expected amplification curves. Amplify the Quality Control DNA at a Cq of approximately 15–22 cycles. Exclude replicates from a triplicate group that are > 0.5 Cq different from the rest of the group.

- Exclude replicates exhibiting abnormal amplification curves. At least two of the three replicates must be included in the final calculation for an individual sample or the qualification process must be repeated for those samples.
- If four or more samples per 10 sample run have replicates removed, repeat the qualification process for all samples.

Performance Characteristics

Table 4 shows the Cq values from gDNA at 0.25 ng/μl from five commercial vendors (B, C, P, R, and T) or extracted from a whole blood sample. NIST reference material at the same concentration is shown for comparison. The Cq values are from three independent operators and three independent qPCR platforms (A, B, S). The results show the average ± standard deviation. Instrument B shows a consistent Cq increase relative to instruments A and S; samples normalized by the Quality Control DNA had consistent dCq values across instruments (data not shown).

Table 4 Cq values of Quality Control DNA Sourced from Vendors or Extracted from Blood

qPCR Instrument	NIST Male 2372	Vendor B	Vendor C	Vendor P	Vendor R	Vendor T	Extracted
Instrument A	18.87 +/- 0.07	19.14 +/- 0.14	18.79 +/- 0.13	19.11 +/- 0.17	19.07 +/- 0.12	19.03 +/- 0.17	18.78 +/- 0.07
Instrument B	20.47 +/- 0.09	20.75 +/- 0.12	20.43 +/- 0.12	20.71 +/- 0.19	20.71 +/- 0.06	20.69 +/- 0.15	20.46 +/- 0.09
Instrument S	19.06 +/- 0.10	19.39 +/- 0.13	18.99 +/- 0.14	19.29 +/- 0.16	19.31 +/- 0.10	19.24 +/- 0.15	19.08 +/- 0.16

Revision History

Document #	Date	Description of Change
Document # 100000031884 v04	August 2021	Updated EU Authorized Representative address. Added Revision History table.

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