RPP30, *EGFP*, *kan* assay for Countable PCR

DESCRIPTION

Why this assay matters

The human *RPP30*, *EGFP*, and *kan* targets are widely used motifs for cell and gene therapy applications. Being able to quickly and confidently detect these sequences saves days of troubleshooting and provides reliable readouts from the very first run.

The Countable *RPP30*, *EGFP*, *kan* assay delivers:

- Rapid setup — pre-verified, ready to use, and compatible with Universal Multiplex (UM) chemistry.

- Flexible format — run as a 3-plex or combine with a user-defined gene of interest (GOI) for flexible multiplexing.

- Consistent performance — sensitive enough to detect rare molecules, precise enough for reproducible results every time.

This assay is part of Countable Labs' Community Assays, a collection of ready-to-run assays shared with the scientific community to accelerate discovery. For the *RPP30*, *EGFP*, *kan* assay, please contact us at hello@countablelabs.com for information on the full primer sequences. All performance data was generated using the Countable platform.

Targets

The *RPP30*, *EGFP*, and *kan* assay is designed to detect *RPP30*, *EGFP*, and *kan* targets with the following specifications on the Countable platform.

Targets	Amplicon length	UM probe
EGFP	98 bp	UM-2
RPP30	81 bp	UM-3
kan	126 bp	UM-4

For Research Use Only. Not for Use in Diagnostic Procedures.

PX00056 | MK033 Rev 1.0   1

Control sample

The following synthetic dsDNA templates spiked into genomic DNA from cell line GM12878 were used to establish the *RPP30*, *EGFP*, and *kan* assay performance and are also recommended to use as a Training Sample during your Countable PCR setup. While not required for running the assay, using this control helps:

- Improve the specificity of your counts

- Serve as a quick check that your setup is performing optimally

- Provide a reference point for monitoring consistency across runs

```
> Template _ sequence _ EGFP
AGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAG
CAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG
CAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAA
> Template _ sequence _ kan
ATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGT
GATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTCTCACCGGAT
TCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTT
```

Assay signal distribution

Clear separation between the target signal and the background is critical for accurate calls. The *RPP30*, *EGFP*, *kan* assay consistently achieves an Intensity Distribution (ID) score above 90 — signifying that the data has a clean distinction between "signal" and "noise," reducing false positives and improving confidence in quantification.
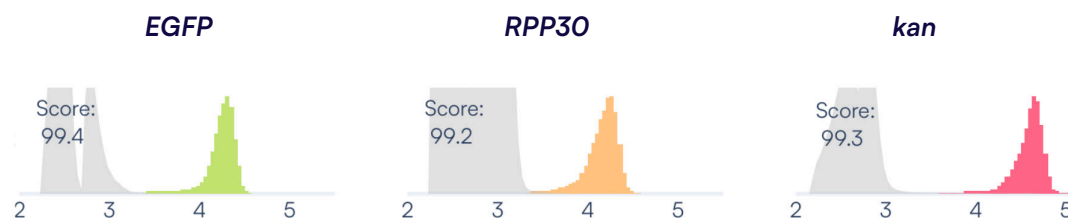

Figure 1. Representative fluorescence intensity histogram for the Countable *RPP30*, *EGFP*, and *kan* assay, showing clear separation between target signal (*EGFP*: green; *RPP30*: orange; *kan*: red) and background (grey). The Intensity Distribution (ID) score for each target appears in the upper-left corner, demonstrating strong signal-to-noise performance.

Instant multiplexing

The *RPP30*, *EGFP*, *kan* assay demonstrates consistent multiplex performance, yielding equivalent Counts per 50 µL whether targets are run individually (1-plex) or together (3-plex). This robust performance saves you time and ensures reliable results when running multiple targets.
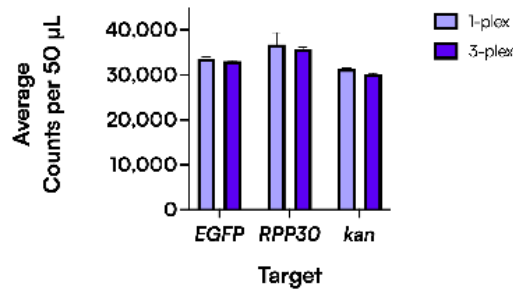

Figure 2. Consistent multiplex assay performance of the Countable *RPP30*, *EGFP*, *kan* assay. Bars represent individual Counts per 50 µL for each reaction, amplified using the synthetic training sample with either a single (1-plex; n=3) or three primer pairs (3-plex; n=3).

Linearity and sensitivity

A dilution series of the control sample shows excellent linearity across a broad dynamic range of counts, demonstrating quantification abilities from high to very low target concentrations.

- This combination enables accurate measurements, whether you're monitoring high-expression constructs or confirming trace residuals.
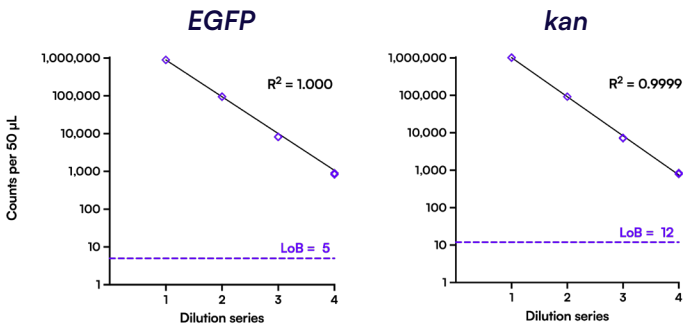

Figure 3. Linearity and limit of blank (LoB) for the Countable *RPP30*, *EGFP*, *kan* assay. A 4-fold dilution series of the synthetic training sample (n=3) shows excellent linearity (R^2 = 1.0000 and 0.9999) across the dynamic range. The dashed line marks the LoB for each target, highlighting the assay's ability to detect even rare targets with accuracy.

Precision

Across the assay's full dynamic range, the %CV remains <5% (*RPP30* %CV = 0.86%), delivering reproducible results run after run. This is essential for comparing results over time, between batches, or across different operators.

Targets	Dilution	1	2	3	4
EGFP	Average counts	894,519	95,226	8,198	853
	% CV	0.39%	0.77%	1.16%	4.63%
kan	Average counts	1,019,672	92,314	7,239	826
	% CV	1.21%	0.55%	1.92%	4.14%

Table 2. *RPP30*, *EGFP*, *kan* assay precision across the dynamic range.

Summary

The *RPP30*, *EGFP*, *kan* assay gives you ready-to-go, high-quality data from the first run — without time lost to assay optimization. This assay has been designed, tested, and shared to help you achieve trustworthy results faster for your QC, R&D, or production monitoring workflows.

RPP30, *EGFP*, *kan* assay

Getting started

This protocol describes the setup and execution of the Countable *RPP30*, *EGFP*, *kan* assay for detecting *RPP30*, *EGFP*, *kan* targets on the Countable PCR platform using Universal Multiplex chemistry.

Detected targets

Targets	Amplicon length	UM probe
EGFP	98 bp	UM-2
RPP30	81 bp	UM-3
kan	126 bp	UM-4

Materials

Listed below are the materials needed for setting up the amplification mix of this specific assay. Refer to Countable PCR™ Reaction Preparation User Guide (IFU004) for the complete list of required materials to set up a Countable PCR reaction.

☐ **4X Countable PCR Mix** (Required)
Cat #: KT0004 (PR0004)

☐ **50X UM-2, UM-3, UM-4 Probes** (Required)
Cat #: KT0005 (PR0007-PR0009)

☐ *RPP30*, *EGFP*, *kan* **50x primer mix** (Required)
Visit website for sequences

☐ **Synthetic training sample** (Optional*)
Visit website for sequences

* The use of a training sample in the Countable system enhances the specificity of counts, verifies assay performance, and can serve as a control, particularly for detecting rare molecules.

Countable PCR reaction set-up

The table below lists the setup of the amplfiication mix specific to this assay. Refer to Countable PCR™ Reaction Preparation User Guide (IFU004) for complete setup instructions.

Reagents	Cat #	Per 50 µL reaction	Final conc.
Nuclease-free water	—	To 50 µL	—
4X Countable PCR Mix	KT0004 (PR0004)	12.5 µL	1X
50X UM-2, -3, -4 Probes	KT0005 (PR0007-9)	1 µL each	1X
50X primer mix*	—	1 µL	1X
Template	—	Variable	—

* Refer to IFU004, Appendix E for details

Thermal cycling conditions

Ensure ramp rate setting of 2 °C/sec. Set the sample volume to 125 µL and the heated lid to 105 °C.

Cycle	Step	Temp (°C)	Time (mm:ss)
1	Initial denaturation	95 °C	02:00
40	Denaturation	95 °C	00:20
	Annealing & extension	55 °C	01:00
1	Hold	12 °C	∞