# MiQuant® CAR T Cell – Lenti, CD19, BCMA and SP

## Determination of Vector Copy Number by dPCR

- ✓ Fast workflow
- ✓ In-Process Control and Release Testing
- ✓ Lentivirus-, CD19- or BCMA- specific target
- ✓ Quantitation of VCN without a standard curve
- ✓ Compatible with QIAcuity platform
- ✓ Accurate and reproducible results
- ✓ Calculation of VCN with analysis software

# MiQuant® CAR T Cell

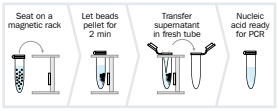
## Principle

The MiQuant® CAR T Cell kits have been developed for the determination of vector copy numbers (VCN) in transduced cells to determine the average copies of integrated lentiviral vectors per cell genome after *ex vivo* transduction of human or other mammalian cells. After extracting genomic DNA from the transduced cells, the copies of the lentiviral gene are determined in relation to the human housekeeping gene PCBP2 by digital PCR. The kits can be used for characterization and release testing of cellular products or as an in-process control in production processes. Different kit versions enable VCN determination of lentivirus-target (works for all lentiviruses) or CD19- or BCMA-specific targets.

### Simple and Fast Workflow

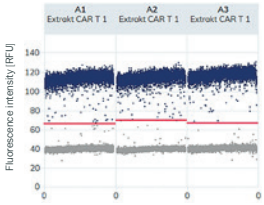
**Sample Preparation**

MiQuant® CAR T Cell – SP is a magnetic bead based extraction kit and has been developed for the isolation of nucleic acids from transduced cells. Extraction process can be done manually or automatically (e.g. with KingFisher™ Flex).




**Digital PCR**

All MiQuant® CAR T Cell kits are freeze-dried and contain all necessary components, including Taq Polymerase, dNTPs, rehydration buffer, and two primer/probe systems. A duplex PCR enables the detection of the human PCBP2 gene (HEX™-labelled probe) and the detection of lentivirus/CD19/BCMA-specific target (FAM™-labelled probe). For quantitation of VCN a standard curve is not required.



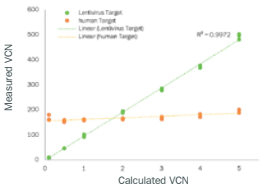

**Analysis**

Calculation of the VCN can be done with the help of an analysis software. By easily uploading the csv.-file, the results from the dPCR [copies/µl] are converted into vector copy numbers taking into account the ploidity and transduction frequency.

**Verification**

MiQuant® CAR T Cell Verification Standards help you to verify the determined VCN. The standards are available for all kit versions (lentivirus, CD19 or BCMA) and contain a target-specific component with a determined VCN and a reference solution standard for diluting the target-specific component (both freeze-dried).*

*Please note, that the MiQuant® CAR T Cell Verification standards are not necessarily required to determine VCN. Quantitation of VCN can be done without a standard curve. Verification standards just help you to verify your results.




## Benchmarking

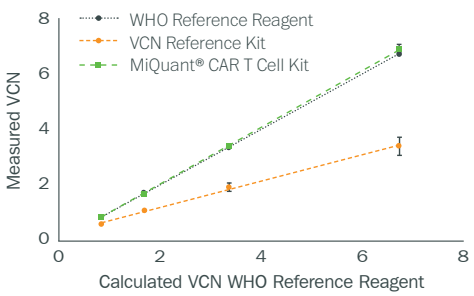



**Fig. 1:** Benchmarking of the MiQuant CAR T Cell Kit with a VCN reference kit determining the VCN of the WHO VCN reference reagent.
The X-axis represents the calculated vector copy number (VCN) for the WHO VCN Reference Reagent and the Y-axis shows the measured VCN of the WHO VCN reference reagent meaured with the respective kits.

## Application Areas

VCN determination with the MiQuant® CAR T Cell kit is useful at different times. During the CAR-T cell production process it can be used as an in-process control to evaluate the transfection efficiency. As part of the QC release testing the VCN must be determined prior to administration to the patient. For long term monitoring of the therapy it is also possible to test blood samples of a patient treated with CAR-T cells. Besides these applications the MiQuant® CAR T Cell kits can also be used for determining VCN in cell culture samples in research and development and are applicable for human or other mammalian cells such as mice or rats.

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