



Orders: 877-616-CELL (2355)
orders@cellsignal.com

Support: 877-678-TECH (8324)

Web: info@cellsignal.com
cellsignal.com

3 Trask Lane | Danvers | Massachusetts | 01923 | USA

Store at +4C
#20418

PathScan® RP G4S Linker/CD3ζ CAR Sandwich ELISA Kit

1 Kit (96 assays)

Species Cross Reactivity:
All

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

Product Includes	Product #	Quantity	Color	Storage Temp
CD3ζ Rabbit mAb Coated Microwells	40560	96 tests		+4C
G4S Linker Rabbit Detection mAb	55714	1 ea	Red (Lyophilized)	+4C
HRP Diluent	13515	5.5 ml	Red	+4C
TMB Substrate	7004	11 ml		+4C
STOP Solution	7002	11 ml		+4C
Sealing Tape	54503	2 ea		+4C
ELISA Wash Buffer (20X)	9801	25 ml		+4C
Cell Lysis Buffer (10X)	9803	15 ml		-20C

Kit contents scale proportionally with size, except sealing tape.

Example: The V1 kit contains 5X the listed quantities above, but will exclude the sealing tape.

The microwell plate is supplied as 12 8-well modules - Each module is designed to break apart for 8 tests.

Description

The rapid protocol (RP) PathScan® RP G4S Linker/CD3ζ CAR Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects scFv-based chimeric antigen receptors (CARs) containing a G4S linker and a CD3ζ signaling domain in a reduced assay time of 1.5 hours. Incubation of cell lysates and detection antibody on the coated microwell plate forms a sandwich with CARs containing a G4S linker in a single step by using one antibody that recognizes CD3ζ and another antibody that recognizes the G4S linker. The plate is then extensively washed and TMB reagent is added for signal development. The magnitude of absorbance for the developed color is proportional to the quantity of scFv-based CARs containing a G4S linker and a CD3ζ signaling domain. Learn more about your ELISA kit options here.

*Antibodies in this kit are custom formulations specific to kit.

Specificity/Sensitivity

The PathScan® RP G4S Linker/CD3ζ CAR Sandwich ELISA Kit detects exogenously expressed levels of scFv-based CARs containing a G4S linker and a CD3ζ signaling domain by using one antibody that recognizes CD3ζ and another antibody that recognizes the G4S linker. The kit sensitivity is shown in Figure 1.

Background

The poly-Glycine-Serine (G4S) linker is a type of flexible, unstructured synthetic peptide linker sequence often leveraged to connect the variable heavy (VH) domain and variable light (VL) domain of single-chain variable fragments (scFvs) and chimeric antigen receptors (CARs) that utilize an extracellular domain scFv for target antigen recognition. The linker itself consists of a core pentapeptide sequence, Gly-Gly-Gly-Gly-Ser, that is repeated and commonly found as either a 15-mer (G4S)₃ or 20-mer (G4S)₄ within scFv-based CARs and scFv fragments. The linker sequence length plays a role in controlling scFv stability and the noncovalent association between the VH and VL domains (1,2).

Background References

- Huston, J.S. et al. (1988) *Proc Natl Acad Sci USA* 85, 5879-83.
- Chen, X. et al. (2013) *Adv Drug Deliv Rev* 65, 1357-69.

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