Note: Calibration curve and quantification should be carried out as described in the user manual.

Cell Extraction Buffer: 50 mM Tris-HCl, pH = 8.0, 150 mM NaCl, 0.5% NP-40 with 0.1% SDS

1. Spin down the plate 5 min @ 2000 xG

Cell Supernatant

2. Transfer 120 µL supernatant to an uncoated microtiter plate
3. Add 80 µL sample dilution buffer (V_f=200 µL), briefly mix on orbital shaker
4. Seal and incubate the plate 60 min @ RT
5. Prewash the wells of the coated microtiter plate 4 times with 350 µL wash buffer.
6. Within 10 minutes of completing the above wash, transfer 150 µL to prewashed coated microtiter plate
7. Continue with "Joint Detection Protocol"

Cell Pellet/Cell Extract

2. Add 200 µL prechilled (4°C) wash buffer and spin 5 min @ 2000 xG
3. Remove wash buffer
4. Lyse cells with 120 µL cell extraction buffer
5. Transfer 80 µL sample to uncoated microtiter plate
6. Add 80 µL sample dilution buffer (V_f=160 µ)
7. Seal and incubate the plate 60 min @ RT
8. Prewash the wells of the coated microtiter plate 4 times with 350 µL wash buffer.
9. Within 10 minutes of completing the above wash, transfer 100 µL to prewashed coated microtiter plate
10. Continue with "Joint Detection Protocol"

Joint Detection Protocol

a. Cover and seal. Incubate 2 hours @ RT w/ intermediate shaking
b. Wash 4 times with 350 µL wash buffer
c. Add 100 µL Biotinylated anti TK1 to each well
d. Cover and seal. Incubate 1 hour @ RT w/ intermediate shaking
e. Wash 4 times with 350 µL wash buffer
f. Add 100 µL streptavidin-HRP conjugate
g. Cover and seal. Incubate 30 minutes @ RT w/ intermediate shaking
h. Wash 4 times with 350 µL wash buffer
i. Add 100 µL TMB substrate to each well. Incubate 15 minutes @ RT **in the dark** with no rotation
j. Add 100 µL Stop solution. Ensure complete mixing
k. Read within 15 minutes @ 450 nm.