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Highly Sensitive Kinase Assays Combining Carna Biosciences QSS Assist™ ELISA Reagents with the Meso Scale Discovery MULTI-ARRAY® Platform

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Abstract

We developed a sensitive kinase assay using a QSS Assist™ ELISA kinase assay kit from Carna Biosciences, Inc. and the detection method from Meso Scale Discovery. The QSS Assist™ ELISA kit is optimized for screening of compounds. Comparing to most other kinase assay kits, QSS Assist ELISA kits are available for both peptide and protein substrates and several ELISA kits developed by Carna Biosciences employ naturally occurring protein substrates, e.g. MAP kinases for MAPK kinases.
Detection of kinase activity is achieved with a primary anti-phospho-serine/threonine or tyrosine antibody and a secondary anti-Ig antibody. Commonly, such secondary antibodies are coupled to horseradish peroxidase (HRP), which allows for signal detection by adding a color reagent, i.e. an HRP substrate which can be detected by measuring optical density. Here we applied an MSD SULFO-TAG® labeled secondary antibody. These antibodies emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® microplates and thus detect the primary antibody with ultimate sensitivity without directly labeling it.

Methods

The TAK1-TAB1 QSS Assist™ assay kit from Carna Biosciences, Inc. has been adapted to the MSD technology. The procedure follows the QSS Assist™ protocol, while largely reducing reagent amounts.
The kinase reaction containing QSS Assist™ kit components (kinase, ATP, GST-tagged substrate, buffer) and the compound to be investigated are incubated in polypropylene plates. After addition of a stop solution, the reaction is transferred to a glutathione coated plate and incubated in order to allow binding of the GST-tagged protein substrate to the plate. After washing and blocking steps, a first anti-phospho S/T antibody is added. After additional incubation and washing the SULFO-TAG® anti-Ig antibody is added. The SULFO-TAG® label emits light upon electrochemical stimulation at the electrode surface of MULTI-ARRAY® or MULTI-SPOT® microplates. Fig 1 shows an illustration of the ELISA assay procedure and principle of MSD detection.

Assay protocol: Kinase reactions were carried out in ***20 µl volume (vs. 40 µl in the original QSS Assist™ manual)*** containing ***0.3 ng TAK1-TAB1 kinase (vs. 1.2 ng)***, 180 nM GST-tagged full-length human MAP2K7 as substrate, 5 µM ATP, 10 mM Mg, 15 mM Tris-HCL (pH 7.5), 0.01% Tween 20, 2 mM DTT and compounds in concentrations ranging from 10 to 0.0001 µM. Incubation in polypropylene 96-well plates for 1h was followed by addition of a stop solution. The assay solution was transferred to glutathione-coated 96-well plates (see below for coating procedure) and incubated for 30-60 min. ***A four times lower amount of 1st antibody solution compared to the original QSS Assist™ manual,*** was added after washing steps and incubated for 30 min. 6 µl of SULFO-TAG® anti Ig antibody were added after additional washing steps and incubated for 30 min, followed by washing steps. MSD Read buffer was added to each well, and signals were detected on the SEKTOR® Imager 6000.
Preparation of glutathione-coated plates: MSD MULTI-ARRAY® microplates were first coated with hemoglobin. Unbound protein was removed by washing and a hetero-bifunctional cross-linker (SSMPB) was coupled on the bound protein. Excess cross-linker was removed by washing and glutathione was coupled through its sulphydryl group to the maleimide group of bound SSMPB. (Murray, A. M. et al., J. Immu. Meth. 218, 133-9, 1998).

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Assay Principle

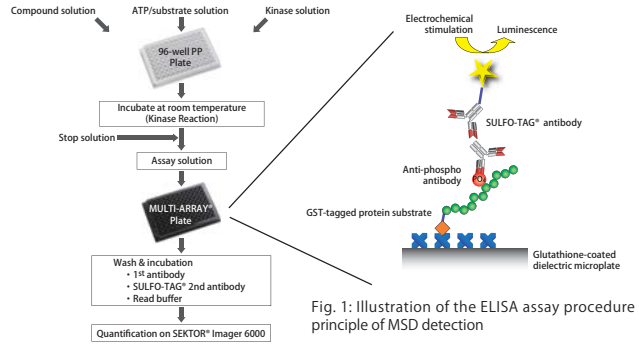

Fig. 1: Illustration of the ELISA assay procedure and principle of MSD detection

Assay Validation

The assay was validated with two known TAK1 inhibitors (Fig. 2)

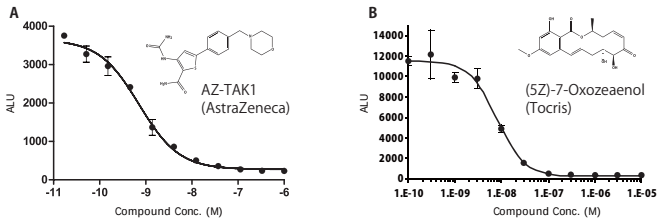

Fig. 2 **A**: AZ-TAK1, a compound of the thiophenecarboxamide class, described by Astra Zeneca. AZ-TAK1 was previously found to inhibit TAK1 with an IC_{50} value of 8 nM (Buglio, D. et al., Blood 120, 347-355, 2012). Here we determined an IC_{50} value of 7.2 nM. **B**: The IC_{50} value of (5Z)-7-oxozeaenol supplied by Tocris Bioscience for TAK1 was determined at 8 nM (Ninomija-Tsuji, J. et al., J Biol Chem 278, 18485-19490, 2003). Here we determined an IC_{50} value of 11.8 nM, which is well in accordance with the reported value.

Conclusion

- **QSS Assist™ ELISA kinase assay kits from Carna Biosciences, Inc. can be adapted to the Meso Scale Discovery MULTI-ARRAY® detection technology**
- **The technology is compatible with a wide range of tagged protein and peptide substrates**
- **We have successfully miniaturized the assay and were able to reduce the materials used from our original protocol to 1/4 (500 dps to 2000 dps)**
- **This new assay technology has been validated with published TAK1 inhibitors from AstraZeneca and Tocris Bioscience**

Carna Biosciences, Inc.

Carna Biosciences offers 110 QSS Assist™ ELISA kinase assay kits (Fig. 3).

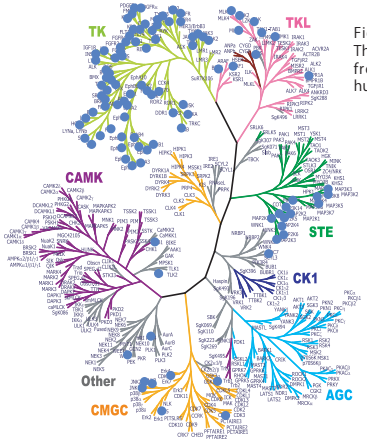

Fig. 3. 110 QSS Assist™ ELISA assays (34 Serine/Threonine and 76 Tyrosine Kinase assays) available from Carna Biosciences are highlighted in the human kinome tree

- **Designed for compound screening, particularly secondary and counter screening**
- **Ready-to-Run, all necessary reagents and a detailed assay protocol is provided**
- **Proteins and peptides as substrates**
- **Designed to be completed within 2-3 h at ATP levels approximating km value**
- **The 1st anti-phospho-antibody is validated and carefully chosen for best signals**