

Product Name

Antibody Based Assay for PAD activity **ABAP**

CAT No.

MQ17.101-96

Size

96 well test

Edition 05.03.2007



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Product Description

The Antibody Based Assay for PAD activity (ABAP) is a solid Enzyme Linked-Immuno-Sorbent Assay (ELISA) for the determination of PAD enzyme activity in cell and tissue lysates. Furthermore it can be used in combination with recombinant PAD enzymes in PAD inhibition assays. Arginin containing peptides have been coated onto strips of a 96-well plate. On each strip one well is coated with a deiminated arginin containing peptide, serving as positive controls. During the first incubation with recombinant PAD enzymes or cell/tissue lysates containing PAD enzymes, arginines are being deiminated. After incubation with PAD containing solutions, the excess enzyme is washed away and a HRP-labelled monoclonal detection antibody specific for deiminated arginine is added to the wells. Development of the plate using HRP substrate results in a staining reaction that is directly proportional to the amount of arginines that have been deiminated. The optical density measured in a plate reader can be directly correlated to the enzyme activity of the control enzyme present in the test, resulting in a quantitation of the PAD enzyme activity.

The ABAP was validated using recombinant human PAD4 (Figure 1) and mouse tissue lysates of a variety of PAD expressing tissues (Figure 2).

Reagents

- **Arginine containing peptides coated 96-well ELISA plate.** The last well of each strip is coated with deiminated arginines containing peptides which serve as positive control in the ABAP test.
- **A ModiQuest Research mouse PAD enzyme** for activity calibration
- **Deimination buffer** (15ml) containing sodium azide as a preservative.
- **Monoclonal anti- deiminated arginin** A proprietary ModiQuest Research HRP-labelled monoclonal antibody specifically binding deiminated arginines

Reagents and equipment required but not provided

- Multiwell plate reader capable of readings at 450nm if using tetramethylbenzamide (TMB) as a substrate. If using a different substrate wavelength should be adjusted accordingly.
- Calibrated adjustable precision pipettes for volumes between 5µl and 1,000µl.
- Plate washer (optional) or manifold dispenser.
- Calibrated beakers and graduated cylinders in various sizes.
- Vortex mixer.
- Wash buffer. (PBS + 0.05% Tween-20)
- Stabilized Chromogen (TMB).
- 2M H₂SO₄ (stop solution)

Precautions and Disclaimer

1. The kit is for R&D use only, not for drug or other uses.
2. As with any product derived from biological sources, proper handling procedures should be used. Please consult the Material safety Data Sheet for information regarding hazards and safe handling practices.
3. The Product may be used with different sample types, therefore each individual laboratory should validate the test system applied.

Storage/Stability

Most of the components of this kit are stable at 4°C. ModiQuest Research mouse PAD enzyme is should be stored at -80°C.

Preparation of the enzym

For activity calibration, the PAD enzym should be diluted at suitable concentrations (for example from 12.5 until 0.1mU) in deimination buffer (40mM Tris-HCl pH7.5; 5mM CaCl₂; 1mM DTT) for the activity curve.

12.5 mU PAD in 100ul deimination buffer per well gives maximum deimination, whereas 0.1mU PAD/well has no detectable activity.

Procedure

1. Preincubate 96-well plate for 30min at 37 °C with 100ul deimination buffer. This step is necessary to equilibrate the ABAP plate.
2. Empty the ABAP plate by inversion and place it on ice. Place also the deimination buffer on ice.
3. Dilute tissue lysates that need to be tested for PAD activity in ice cold deimination buffer. Do the same with the PAD activity calibration samples. Total volume that should be added in each well is 100µl (It is recommended that different concentrations of lysates are tested. Usually 1µl tissue lysate in 100µl deimination buffer will be sufficient)
4. Add all samples in the ABAP plate while keeping everything on ice.
5. Incubate the ABAP plate for 1hour and 15 minutes at 37 °C in a humidified chamber.
6. Wash the plate 5 times with wash buffer.
7. Dilute the ModiQuest Research HRP-labelled monoclonal antibody in washbuffer + 1%BSA at a concentration of 1µg/ml. Add 100µl antibody solution in each well.
8. Incubate 1 hour at 37 °C in a humidified chamber.
9. Wash the plate 5 times with wash buffer.
10. Add 100µl TMB substrate in each well and let the blue color develop. (room temperature).
11. Add 100µl 2M H₂SO₄ in each well to stop the reaction. The blue color will now change to yellow.
12. Read OD at 450nm in a multiwell plate reader.

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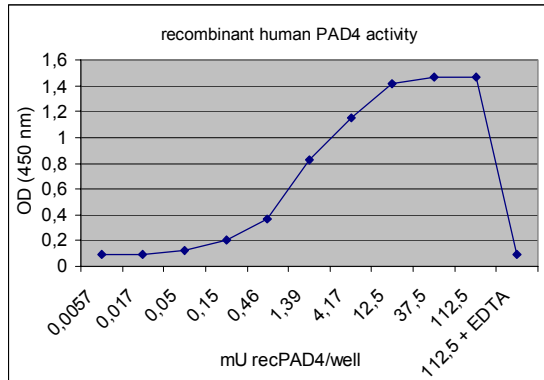


Figure 1: Increasing amount of recombinant human PAD4 have been added to ABAP-plates coated with arginine containing peptides, resulting in the detection of increasing amounts of citrulline-peptides. Addition of EDTA abrogates PAD activity through the capture of calcium ions.

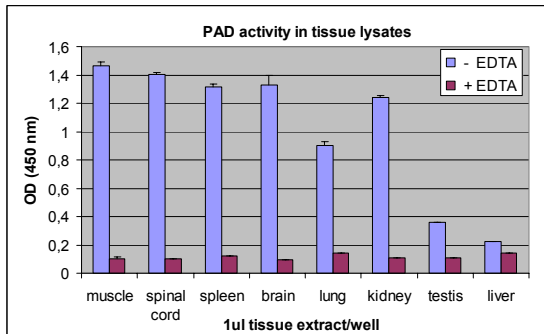


Figure 2: Detection of PAD activity in different mouse tissue samples. One microliter of tissue lysates were used in either a calcium-containing (- EDTA) or a EDTA-control (+ EDTA) reaction and PAD activities were monitored by standard ABAP procedures.