Product Name
Antibody Based Assay for PAD activity (ABAP)

CAT No.
MQ 17.101-96

LOT No.
18307

Quantity
96 well test
Edition: June 24, 2019

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Disclaimer
The kit is for R&D use only. NOT for use in diagnostic or therapeutic procedures.
A license from ImmunoPrecise Antibodies is required for use outside the research field.

Product Description
The Antibody Based Assay for PAD activity (ABAP) is a solid Enzyme-linked immunosorbent 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. Arginine containing peptides have been coated onto strips of a 96-well plate. On each strip, well H is coated with a deiminated arginine-containing peptide, serving as a positive control. 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 monoclonal detection antibody specific for deiminated arginine is added to the wells. After incubation wells are washed and incubated with a HRP-labelled polyclonal anti-mouse immunoglobulin antibody. 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
- **ABAP 96-well plate.** Well H of each strip is coated with a deiminated arginine-containing peptide, which serves as a positive control in the ABAP test. All other wells are coated with arginine containing peptides.
- **Human PAD enzyme** for activity calibration (human PAD enzyme is purified from bacterial cell lysates).
- **Deimination buffer** containing sodium azide as a preservative.
- **Mouse anti-deiminated arginine**. A proprietary ModiQuest Research mouse monoclonal antibody specifically binding deiminated arginines.
- **HRP-labelled anti-mouse Ig antibody.**

Reagents and equipment required, but not provided
- Multiwell plate reader capable of readings at 450 nm if using Tetramethylbenzidine (TMB) as a substrate. If using a different substrate, wavelength should be adjusted accordingly.
- Calibrated adjustable precision pipettes for volumes between 5 µl and 1000 µl.
- Plate washer (optional) or manifold dispenser.
- Humidified chamber.
- Calibrated beakers and graduated cylinders in various sizes.
- Vortex mixer.
- Wash buffer (PBS + 0.05% Tween-20).
- Bovine Serum Albumin (BSA).
- Stabilized Chromogen (TMB).
- 2M H_2SO_4 (stop solution).

Precautions
1. For professional users.
2. As with any product derived from biological sources, proper handling procedures should be used.
3. The product may be used in combination with different sample types and materials, therefore each individual laboratory should validate the applied test system.

Storage/Stability
- **Human PAD enzyme**
  Store at -80°C. After use, immediately refreeze at -80°C.
  Avoid repeated freeze-thaw cycles. Keep the enzyme on ice all time.
- **ABAP 96-well plate**
  Store at -20°C.
  Avoid repeated freeze-thaw cycles.
- **Mouse anti- deiminated arginine**
  Store at -20°C. After first time use, store at 4°C.
  Avoid repeated freeze-thaw cycles.
- **HRP-labelled anti-mouse Ig**
  Store at -20°C. After first time use, store at 4°C.
  Avoid repeated freeze-thaw cycles.
- **Deimination buffer**
  Store at -20°C.

Preparation of the enzyme
Spin down the vial containing the MQR human PAD enzyme at 4°C before opening. For activity calibration, the PAD enzyme should be diluted at suitable concentrations (for example from 2.0 until 0.002 mU) in deimination buffer (40 mM Tris-HCl pH 7.5; 5 mM $CaCl_2$; 1 mM DTT) for the activity curve.
2.0 mU PAD in 100 µl deimination buffer per well gives maximum deimination, whereas <0.002 mU PAD/well has no detectable activity.

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Procedure

1. Preincubate 96-well plate for 30 minutes at 37°C with 100 µl deimination buffer. This step is necessary to equilibrate the ABAP plate.
2. Empty the ABAP plate by inversion and place it on ice. In addition, place 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 100 µl (It is recommended that different concentrations of lysates are tested. Usually 1 µl of tissue lysate in 100 µl deimination buffer will be sufficient)
4. Add all samples in the ABAP plate while keeping everything on ice.
5. Incubate the ABAP plate for 1 hour and 15 minutes at 37°C in a humidified chamber.
6. Wash the plate 5 times with wash buffer.
7. Dilute the MQR mouse anti-deiminated arginine antibody 1:1000 in wash buffer + 1% BSA. Add 100 µl antibody solution to each well.
8. Incubate the ABAP plate for 1 hour at 37°C in a humidified chamber.
9. Wash the plate 5 times with wash buffer.
10. Dilute the HRP-labelled anti-mouse Ig antibody 1:2000 in wash buffer + 1% BSA. Add 100 µl antibody solution to each well.
11. Incubate the ABAP plate for 1 hour at 37°C in a humidified chamber.
12. Wash the plate 5 times with wash buffer.
13. Wash the plate 3 times with wash buffer without Tween-20.
14. Add 50 µl TMB substrate in each well and let the blue colour develop (at room temperature in the dark).
    Please note: Fast development time possible.
15. Add 50 µl 2M H_2SO_4 in each well to stop the reaction. The blue colour will now change to yellow.
16. Read OD at 450 nm in a multiwell plate reader.

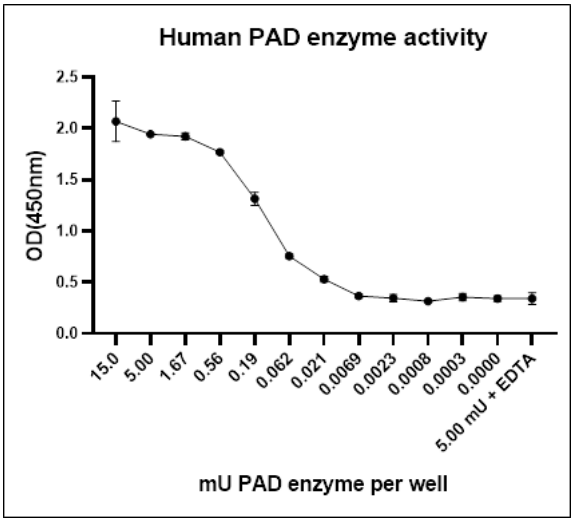

Figure 1: Increasing amounts of recombinant human PAD enzyme have been added to ABAP-plates coated with arginine-containing peptides, resulting in the detection of increasing amounts of citrulline-containing peptides. Addition of EDTA abrogates PAD activity through the capture of calcium ions.

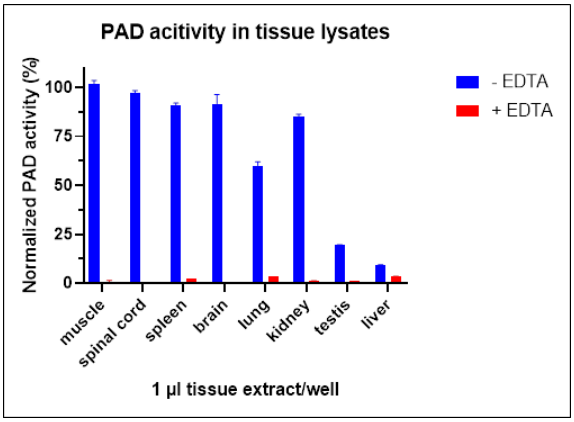

Figure 2: Detection of PAD activity in different mouse tissue samples. One microliter of tissue lysate was used in either a calcium-containing (- EDTA) or a calcium-depleted (+ EDTA) control reactions and PAD activities were monitored by standard ABAP procedures. Tissue lysate PAD activities were normalized to maximum recombinant human PAD activity (- EDTA) and inhibited human PAD (+ EDTA). Please note: This is one representative picture showing ABAP assay capability.