

**Product Name**  
recombinant human Peptidyl Arginine Deiminase  
4 (hPAD4)

**CAT No.**  
MQ 16.203-2.5

**LOT No.**  
0909-15

**Quantity**  
2.5 Units

Edition: November 2, 2015

#### Intended use

This product is for research use only. NOT for use in diagnostic or therapeutic procedures.

Recombinant human PAD4 is intended for studies on its deimination properties.

#### Reagent provided

Solution in 20mM Tris-HCl, pH 7.6, containing 10mM  $\beta$ -mercaptoethanol, 400mM NaCl, 250mM Imidazole, 1mM EDTA and 10% glycerol.

#### Unit Definition

One unit will produce 1 $\mu$ mole of N- $\alpha$ -benzoylcitrulline ethyl ester from BAEE per hour at 55°C at pH 7.2.

#### Activity

Activity has been tested in an MQR Antibody Based Assay for PAD activity (ABAP) (Cat. no. MQ-17.101) and compared to rabbit skeletal muscle PAD from Sigma-Aldrich (figure 1).

#### Purity

Recombinant human PAD4 comes with a HIS-tag and has been purified from bacterial cell lysate using Ni<sup>2+</sup>-beads..

#### 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 different techniques and in combination with different sample types and materials, therefore each individual laboratory should validate the applied test system.

#### Storage instructions

Store at -80°C.

**\*AVOID FREEZE THAW\***

#### Deimination protocol

##### Deimination of coated proteins:

Dilute the enzyme in deimination buffer (40mM Tris-HCl pH7.5; 5mM CaCl<sub>2</sub>; 1mM DTT).

Recommended PAD concentration: 0.5 - 12.5mU enzyme in 100ul deimination buffer if used in an ELISA based assay using 96-well plates.

Deiminate 1.5 hours at 37°C.

##### Deimination of proteins in solution:

The protein(s) to be deiminated should be dissolved or diluted in deimination buffer (0.1M Tris-HCl pH7.5; 10mM CaCl<sub>2</sub>; 5mM DTT) at a concentration of 1mg/ml.

Add PAD enzyme to the protein solution (50mU PAD enzyme per 100 $\mu$ g protein) and incubate 2h at 37°C.

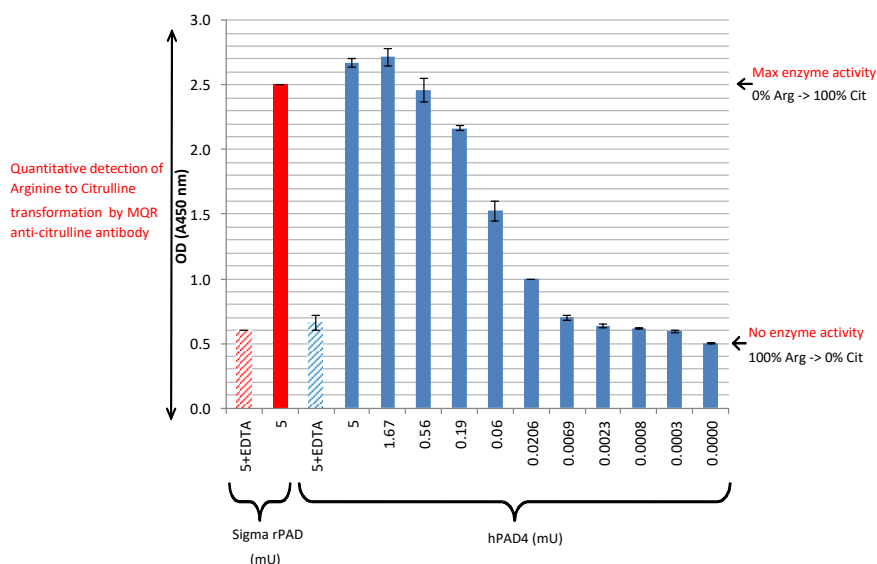


Figure 1: ABAP 96-well strips has been used which are coated with arginine containing peptides. Dilution series was prepared from rec.hu.PAD4. Plates were incubated for 1h and 15 min at 37°C with the enzyme. Deiminated arginines were detected with a proprietary MQR anti-citrulline antibody. EDTA was added in the wells with the highest concentration of enzyme to show its specificity since PAD enzymes are Ca<sup>2+</sup> dependent. Rabbit skeletal muscle PAD from Sigma-Aldrich (Catalogue no: P1584) has been used at a concentration of 0.5 - 12.5mU/well as a reference which gives maximum deimination.