Product Name
Recombinant human Peptidyl Arginine Deiminase 2 (hPAD2)

CAT No.
MQ16.201-2.5

LOT No.
0731-15

Size
2.5 Units

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Intended use
This product is for research use only. NOT for use in diagnostic or therapeutic procedures.
Recombinant human PAD2 is intended for studies on its deimination properties.

Reagent provided
Solution in 20mM Tris-HCl, pH 7.6, containing 10mM β-mercaptoethanol, 400mM NaCl, 250mM Imidazole, 1mM EDTA and 10% glycerol.

Unit Definition
One unit will produce 1µmole of N-α-benzoylcitrulline ethyl ester from BAEE per hour at 55ºC at pH 7.2.

Activity
Activity has been tested in an MQR Antibody Based Assay for PAD activity (ABAP) (Cat. no. MQ17.101) and compared to rabbit skeletal muscle PAD from Sigma-Aldrich (Figure 1).

Purity
Recombinant human PAD2 includes a HIS-tag and has been purified from bacterial cell lysate using Ni^{2+}-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 -80ºC.
AVOID FREEZE-THAW CYCLES

Deimination protocol
Deimination of coated proteins:
Dilute the enzyme in deimination buffer (40mM Tris-HCl pH7.5; 5mM $CaCl_2$; 1mM DTT).
Recommended PAD concentration: 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 $CaCl_2$; 5mM DTT) at a concentration of 1mg/ml.
Add PAD enzyme to the protein solution (50mU PAD enzyme per 100µg protein) and incubate 2h at 37°C.

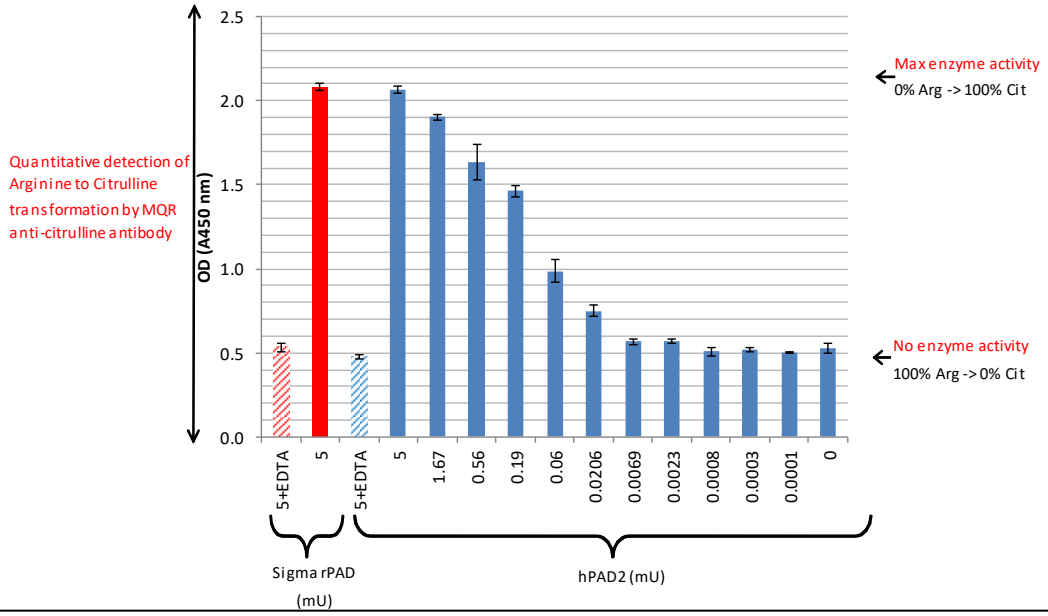

Figure 1: ABAP 96-well strips have been used, which are coated with arginine-containing peptides. Dilution series were prepared from rec. hPAD2. Plates where incubated for 1h and 15 min at 37ºC with the enzyme. Deiminated arginines were detected with a proprietary MQR anti-citrulline antibody. EDTA was added to the wells, which contained a high concentration of enzyme to show its specificity since PAD enzymes are Ca^{2+} dependent. Rabbit skeletal muscle PAD from Sigma-Aldrich (Catalogue no: P1584) has been used at a concentration of 20mU/well as a reference, which gives maximum deimination.

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