

**PAD4 (Peptidyl arginine deiminase, type IV)**

**CATALOG NO.:** PAD-21-479

**LOT NO.:**

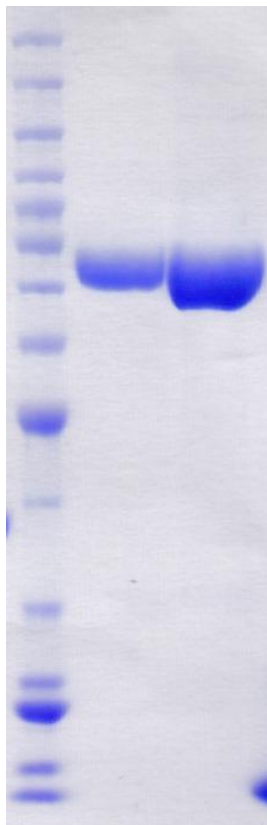
**DESCRIPTION:** Human recombinant PAD4 (residues 2-663; NCBI Reference Sequence NP\_036519.2; MW = 76.5 kDa) expressed in *sf21* insect cells with an N-terminal Strep-tag. PAD4 (PADI4) catalyzes the hydrolytic deimination of specific arginine residues to citrulline and ammonia in a calcium-dependent manner.

**PURITY:** >95% by SDS-PAGE

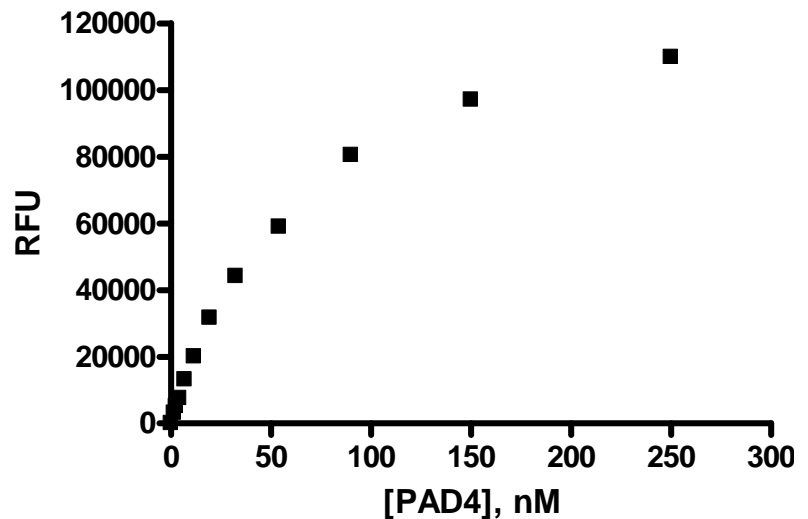
**ASSAY CONDITIONS:** RBC's PAD4 displays deiminase activity in an endpoint OPA/DTT coupled reaction with BAEE substrate. Reactions containing variable PAD4 with 1mM N- $\alpha$ -benzoyl-L-arginine ethyl ester (BAEE) and 200 $\mu$ M CaCl<sub>2</sub> in 100mM HEPES, pH 8, 50mM NaCl, 2mM DTT, 0.6 mg/mL BSA, 1% DMSO were incubated at room temperature for 60 minutes. Fluorescence signal was read (Ex 413nm/Em 476nm) after 60 minute incubation with quench/detection buffer containing 7.5mM o-phthaldialdehyde, 50mM EDTA and 7.5mM DTT (see figure below).

**SUPPLIED AS:** \_\_\_  $\mu$ g/ $\mu$ L in 20 mM Tris, pH 8, 500 mM NaCl, 10% (v/v) glycerol, 1mM EDTA, 2mM DTT as determined by OD<sub>280</sub>.

**STORAGE:** -70°C. Thaw quickly and store on ice before use. The remaining, unused, undiluted enzyme should be snap frozen, for example in a dry/ice ethanol bath or liquid nitrogen. Minimize freeze/thaws if possible, but very low volume aliquots (<5  $\mu$ l) or storage of diluted enzyme is not recommended.



Coomassie blue stained SDS-PAGE (4-20% acrylamide) of 4 and 10 $\mu$ g of RBC PAD4. MW markers (left) are, from top, 220, 160, 120, 100, 90, 80, 70, 60, 50, 40, 30, 25, 20, 15, 10 kDa.



**Deiminase Activity of PAD4.** Deimination of BAEE was determined by quantification of ammonia generation using OPA/DTT detection. The 30 $\mu$ L reaction contained 1mM BAEE, 200 $\mu$ M CaCl<sub>2</sub> and variable amount of PAD4. After incubation at room temperature for 60 minutes, the reaction was quenched by the addition of 50mM EDTA, 7.5mM OPA and 7.5mM DTT. The resulting fluorescence (ex. 413nm/em. 476nm) was measured using CLARIOstar Reader (BMG Labtech)

This product is NOT intended for therapeutic or diagnostic use in animals or in humans.