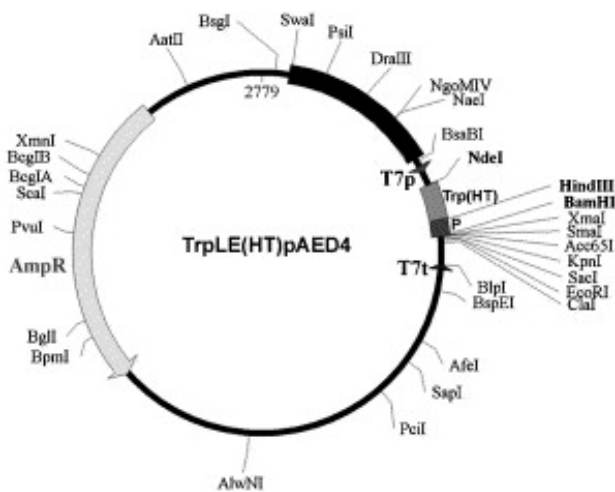
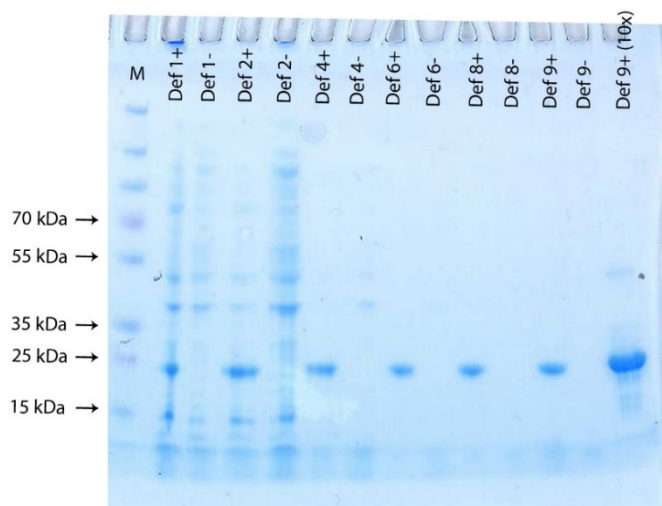


## Detection kit for autoantibodies against enteric alpha-defensins

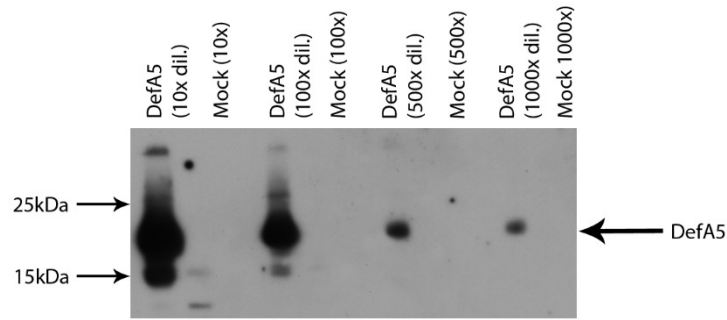
Human enteric alpha-defensin HD5 has been recently identified by our laboratory as a novel and clinically relevant autoantigen in autoimmune disease called APECED (**Dobes et al, 2015, Gastroenterology 149:139-50**). Since up to 30% of APECED patients are seropositive for autoantibody to HD5, its detection could serve as a valuable marker for ongoing intestine autoimmune process. In order to provide a tool for experimental and clinical detection of serum anti-HD5 autoantibody, we cloned, expressed and tested recombinant HD5 (**rHD5**) for its specificity and sensitivity towards serum isolated from anti-HD5 seropositive patients. HD5 gene was amplified and cloned into TrpLE(HT)pAED4 vector (**Fig. 1**) and its product was expressed, isolated and detected by Coomassie stained gel (**Fig. 2**). Subsequent experiments confirmed that rHD5 can be very sensitively recognized by a commercially available antibody at high dilution rate (**Fig. 3**), as well as by defensin-seropositive sera from APECED patients (**Fig. 4 and 5**) but not by a serum from a healthy control (**Fig. 4**).



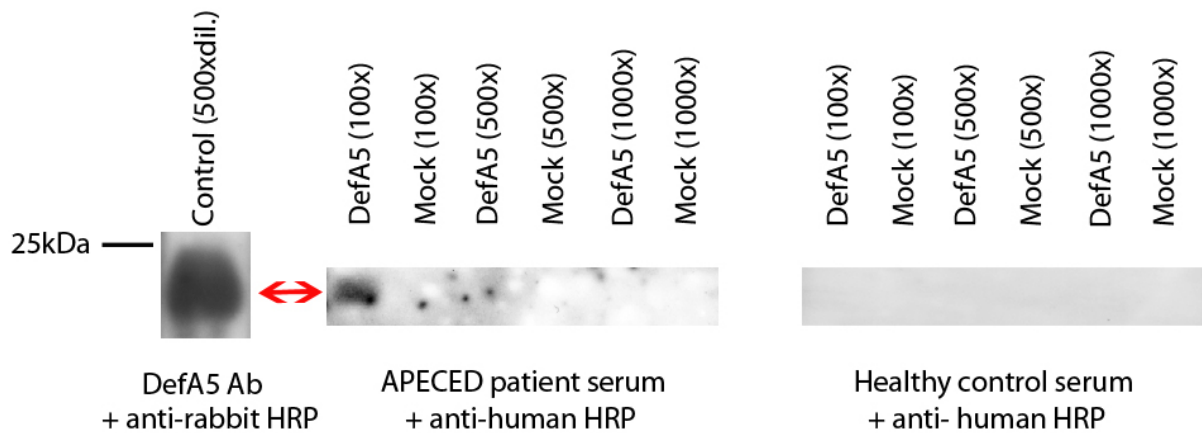
**Fig.1.** Schematic representation of the expression vector pAED with inserted sequences coding for a portion of the tryptophan operon of *E. coli* (*trp* ΔLE 1413) and the (His)<sub>6</sub> affinity-tag (labeled “Trp(HT)”). P (located between the *Hind*III and *Bam*HI sites) indicates the position of the DNA inserts coding for the mature α-defensins. (M Pazgier, J Lubkowski, Expression and purification of recombinant human α-defensins in *Escherichia coli*, *Protein Expr Purif.* 2006 Sep;49(1):1-8.)



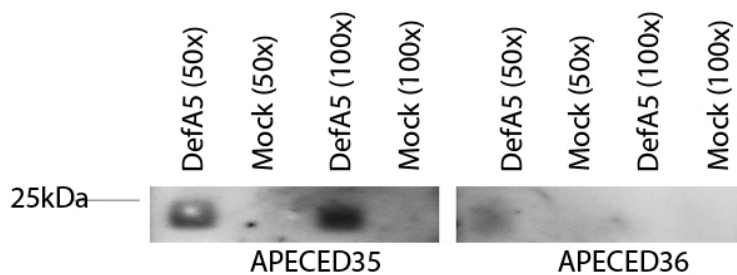
**Fig.2.** Coomassie blue gel staining of DEFA5 protein isolated from *E.coli* after Isopropyl β-D-1-thiogalactopyranoside (IPTG) induction. Def1+ to Def9+ represents the first to ninth fraction of TrpLE(HT)pAED4-DefA5 during releasing protein from inclusion bodies. Def1- to Def9- represents the empty vector TrpLE. Def9+ (10x) represents the final TrpLE(HT)pAED4-DefA5 isolation and was loaded in 10x higher concentration compared to other fractions.



**Fig.3.** Western blot of DEFA5 protein (23kDa) isolated from E.coli after (IPTG induction and after releasing it from inclusion bodies of bacteria E.coli. Gel was stained with anti-DEFA5 rabbit polyclonal antibody (Abnova) and anti-rabbit-HRP secondary antibody.



**Fig.4.** Western blot of APECED patient serum vs. healthy control serum which were tested for presence of autoreactive antibodies against human DEFA5 protein.



**Fig.5.** Western blot of APECED patient serum #35 and #36 which were tested for presence of autoreactive antibodies against human DEFA5 protein.

**Conclusions:** The results show that newly prepared recombinant human enteric defensin HD5 (rHD5) is a suitable substrate to detect the presence of autoantibodies in patients suffering from APECED or potentially also from other types of intestine-related autoimmunity. The construction of ELISA HD5-specific autoantibody detection kit is currently under construction.

To get more information about the rHD5 or to buy nonexclusive licence for its production, please contact Center for Technology Transfer, IMG AS CR, Videnska 1083, 14220 Praha 4, Czech Republic; Tel. (420-241 063 227 or 420-602 892 876).