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▣ BRIEF COMMUNICATION

# Specific Detection of *Acanthamoeba* species using Polyclonal Peptide Antibody Targeting the Periplasmic Binding Protein of *A. castellanii*

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**Abstract:** *Acanthamoeba* keratitis (AK) is a rare ocular disease, but it is a painful and sight-threatening infectious disease. Early diagnosis and adequate treatment are necessary to prevent serious complications. While AK is frequently diagnosis via several PCR assays or *Acanthamoeba*-specific antibodies, a more specific and effective diagnostic method is required. This study described the production of a polyclonal peptide antibody against the periplasmic binding protein (PBP) of *A. castellanii* and investigated its diagnostic potential. Western blot analysis showed that the PBP antibody specifically reacted with the cell lysates of *A. castellanii*. However, the PBP antibody did not interact with human corneal epithelial (HCE) cells and the other 3 major causative agents of keratitis. Immunocytochemistry (ICC) results revealed the specific detection of *A. castellanii* trophozoites and cysts by PBP antibodies when *A. castellanii* were co-cultured with HCE cells. PBP antibody specificity was further confirmed by co-culture of *A. castellanii* trophozoites with *F. solani*, *S. aureus*, and *P. aeruginosa* via ICC. The PBP antibody specifically reacted with the trophozoites and cysts of *A. polyphaga*, *A. hatchetti*, *A. culbertsoni*, *A. royreba*, and *A. healyi*, thus demonstrated its genus-specific nature. These results showed that the PBP polyclonal peptide antibody of *A. castellanii* could specifically detect several species of *Acanthamoeba*, contributing to the development of an effective antibody-based AK diagnostics.

Key words: *Acanthamoeba* keratitis, periplasmic binding protein, peptide antibody, species specificity

*Acanthamoeba* spp. are widely distributed in the environment, and they are causative agents of several amoebic diseases such as granulomatous amebic encephalitis (GAE) and amoebic keratitis [1,2]. *Acanthamoeba* keratitis (AK) is a rare but painful and severe corneal infection found in contact lens wearers [1-3]. Unfortunately, diagnosis of AK is difficult and often delayed. For successful AK treatment, rapid and accurate diagnostic modalities are highly required.

Most cases of AK have been diagnosed by corneal scraping, polymerase chain reaction (PCR), in vivo confocal microscopy, and impression cytology [4]. Corneal scraping culture to confirm the presence of *Acanthamoeba* is currently the standard for AK diagnosis, but it takes several days to acquire a positive result [4]. To enable rapid and accurate diagnosis of AK, new PCR-based methods [5-8] and *Acanthamoeba*-specific antibody-based diagnosis [9-13] have been studied. AK is often reported as a mixed infection with viral, bacterial, or fungal pathogens [14,15]. Since the initial signs and symptoms of AK are similar to those of other corneal pathogens, mixed infections should be considered for accurate AK diagnosis [4]. There is an urgent need to search the differential diagnosis of AK in the mixed infection state.

In a previous study, periplasmic binding protein (PBP) was upregulated in the pathogenic *A. castellanii* strain than in the non-pathogenic *A. castellanii* strain [16]. PBP is a protein involved in cellular uptake and chemotaxis, which are found in various members of the domains, Bacteria and Archaea [17], but its role in *Acanthamoeba* is not clear. PBP has recently been investigated as a new class of biorecognition elements in a competitive enzyme-linked immunosorbent assay [18]. PBP-based magnetic beads were used to isolate and detect thiamine from complex biological matrices of fish eggs [19]. To specifically

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distinguish *Acanthamoeba* spp. from multiple etiologies of keratitis, we produced an *Acanthamoeba*-specific polyclonal peptide antibody against PBP of *A. castellanii* and evaluated its diagnostic potential.

Human corneal epithelial (HCE) cells (ATCC PCS-700–010) were cultured at 37℃ with 5% $CO_2$ atmosphere in endothelial cell growth medium kits (KGM BulletKit) (Lonza, Portsmouth, New Hampshire, USA). *A. castellanii* (ATCC 30868) trophozoites were cultured in Peptone-Yeast-Glucose (PYG) media at 25℃ with cysts being induced in encystment media at 25℃. *A. polyphaga*, *A. hatchetii*, *A. culbertsoni*, *A. royreba*, and *A. healyi* were kindly provided by Prof. Ho-Joon Shin (Ajou University, Suwon, Korea). *Fusarium solani* (NCCP 32678) was cultured in Sabouraud Dextrose (SD) media at 25℃, while *Pseudomonas aeruginosa* (NCCP 16091) and *Staphylococcus aureus* (NCCP 15920) were cultured in Brain Heart Infusion (BHI) media at 37℃.

The PBP of *A. castellanii* consists of 1,761 bp and encodes 586 amino acids with a calculated mass of 64.46 kDa (GeneBank accession No. MW683235.1). To design a peptide antigen with optimal antigenicity, amino acid sequences of PBP of *A. castellanii* were compared with that of *F. albosuccineum* (Fa_PBP, KAF4442825.1), *S. aureus* (Sa_PBP, BBA23260.1), and *P. aeruginosa* (Pa_PBP, KJJ10303.1) (Fig. 1A). Amino acid sequence homology results revealed that PBP of *A. castellanii* had 21.5%, 20.3%, and 26.4% similarity with that of *F. albosuccineum*, *S. aureus*, and *P. aeruginosa*, respectively. The amino acids in the boxed area in Fig. 1A were selected for peptide antibody production using the peptide prediction software (AbFRONTIER, Seoul, Korea). We used RoseTTAFold to generate a 3-dimen-

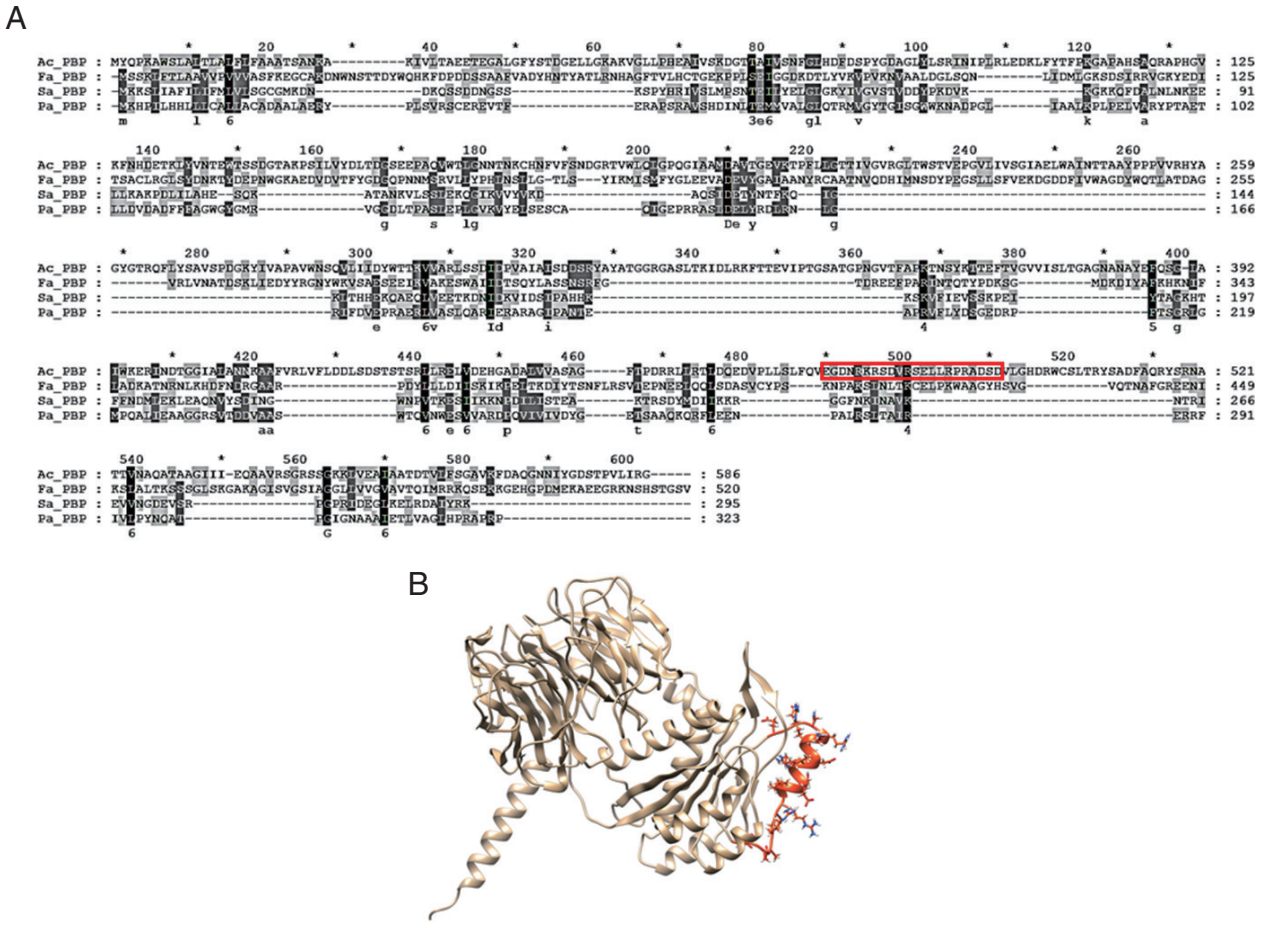


**Fig. 1.** Analysis of amino acid sequence and 3-dimensional conformation of periplasmic binding protein (PBP). (A) Multiple amino acid sequence alignment was produced using the CLUSTAL_X version 2.1. Aligned PBP amino acid sequences of *A. castellanii* (Ac_PBP, QVH35977.1), *F. albosuccineum* (Fa_PBP, KAF4442825.1), *S. aureus* (Sa_PBP, BBA23260.1), and *P. aeruginosa* (Pa_PBP, KJJ10303.1) were compared. Conserved regions were denoted with black shading and the boxed sequence was used to raise the anti-PBP polyclonal peptide antibody. (B) The 3-dimensional structure of PBP was predicted by RoseTTAFold software.

sional model for conserved domains from the protein [20]. The RoseTTAFold predicted the entire structure of the PBP, and the amino acid sequence corresponding to the epitope portion is highlighted in red (Fig. 1B). Based on this illustration, we speculate that this antigenic site would serve as a good antigenic epitope as it is easily exposed to the outer surface.

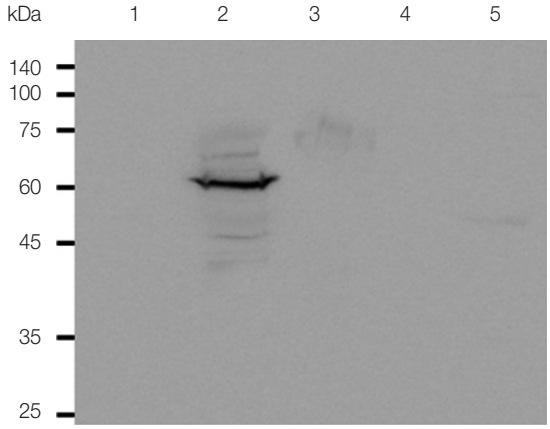


**Fig. 2.** Specificity of the anti-periplasmic binding protein (PBP) of *A. castellanii* was determined by western blot analysis using cell lysates from different organisms. Lane 1; HCE cells, Lane 2; *A. castellanii*, Lane 3; *F. solani*, Lane 4; *S. aureus*, Lane 5; *P. aeruginosa*.

The peptide sequence of PBP (C-EGDNRKRSDVRSELLRPRADSD) used as the immunogen and the antibody raised against the peptide were purchased from AbFRONTIER [13]. To investigate the specificity of the PBP antibody, western blotting was conducted using 20 μg of HCE cells, *A. castellanii*, *F. solani*, *S. aureus*, and *P. aeruginosa* lysates. Cell lysates were resolved on 10% SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was blocked with 5% skim milk in Tris buffered saline containing 0.05% Tween 20 (TBST) for 2 h and incubated overnight at 4˚C with the PBP antibody (1:1,000 dilutions in 5% skim milk). The membrane was incubated with HRP-conjugated anti-rabbit IgG (Sigma-Aldrich, St. Louis, Missouri, USA) (1:5,000 dilutions) for 1 h at room temperature (RT). Reactive bands were developed using Clarity Enhanced Chemiluminescence reagent (Thermo Fisher, Waltham, Massachusetts, USA). As shown in Fig. 2, the PBP antibody showed a strong reactive signal with *A. castellanii*, while immunoreactions were not observed with HCE cells and other causative agents of keratitis.

To confirm the specificity of the PBP antibody, immunocy-

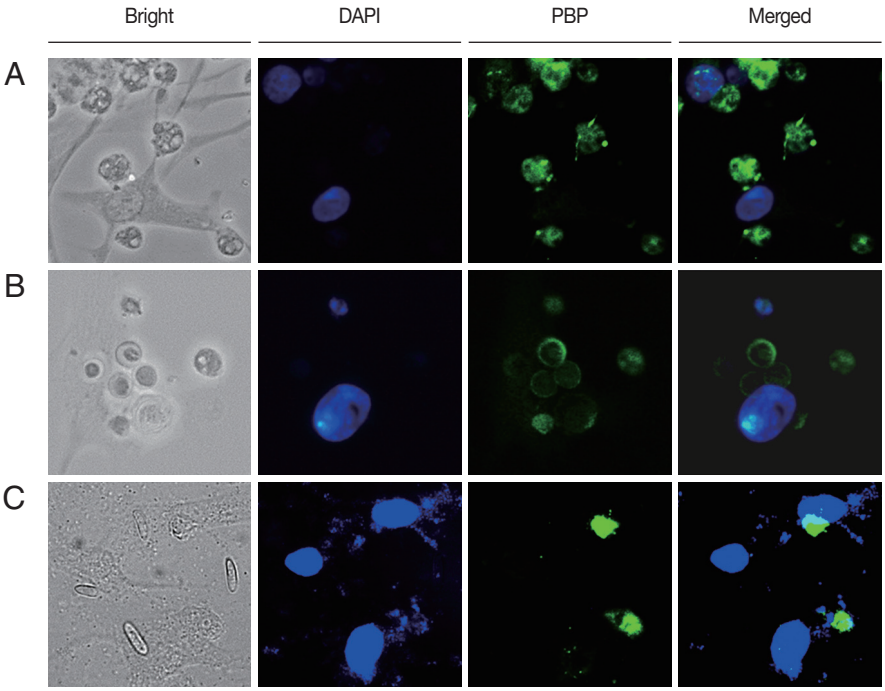


**Fig. 3.** Immunocytochemical staining using anti-PBP antibodies. Human corneal epithelial (HCE) cells and *A. castellanii* trophozoites (A) and cysts (B) were co-cultured. *F. solani*, *S. aureus*, and *P. aeruginosa* were inoculated into the cultures (*A. castellanii* trophozoites and HCE cells) and incubated for 1 h (C). The co-cultured cells were observed under a fluorescent microscope. Bright-field, DAPI staining (blue), PBP antibody combined with CFL488-conjugated secondary antibody (green), and merged images were acquired at 400× magnification.

**Table 1.** Specific detection of five reference *Acanthamoeba* spp. by immunocytochemistry assay

| *Acanthamoeba* spp. | Morphological types | Amoebic disease | Immunocytochemistry | |
|---|---|---|---|---|
| | | | Trophozoites | Cysts |
| *A. polyphaga* | Group II | AK | ± | ± |
| *A. hatchetti* | Group II | AK | + | + |
| *A. culbertsoni* | Group III | AK, GAE | + | + |
| *A. royreba* | Group III | AK | + | + |
| *A. healyi* | Group III | GAE | + | + |

tochemical staining was performed using *A. castellanii* trophozoites and cysts co-cultured with HCE cells. HCE cells ($3 \times 10^5$ cells) were cultured on sterile cover glass in a 6-well plate. The following day, they were co-cultured with *Acanthamoeba* trophozoites ($5 \times 10^5$ cells) and cysts ($5 \times 10^5$ cells) for 5 h at 37℃ in a 5% $CO_2$ incubator. The cells were fixed with 100% methanol for 5 min and subsequently permeabilized with PBST for 10 min at RT. The cells were subsequently blocked using blocking buffer (1% bovine serum albumin and 22.52 mg/ml glycine in PBST) for 30 min at RT. The cells were incubated overnight at 4℃ with 1:200 diluted PBP antibody in blocking buffer and probed with CFL-488 fluorophore-conjugated anti-rabbit IgG antibody (1:400 dilutions) (Sigma-Aldrich) for 1 h at RT. After washing, cells were stained with VECTASHIELD mounting medium with 4'6-diamidino-2-phenylindole (DAPI) (Abcam, Burlingame, California, USA) and observed under a fluorescent microscope (Leica DMi8, Wetzlar, Germany). *A. castellanii* trophozoites (Fig. 3A) and cysts (Fig. 3B) showed strong immunoreactive signal with the PBP antibody (green). However, HCE cells did not show any reactive signal with the PBP antibody while the HCE cell nuclei were counterstained with DAPI (Fig. 3A, B). Additionally, a specific reaction of PBP antibody for *A. castellanii* was observed from the co-cultured cells of *F. solani*, *S. aureus*, and *P. aeruginosa*. HCE cells and trophozoites of *A. castellanii* were co-cultured with *F. solani*, *S. aureus*, and *P. aeruginosa* for 1 h. PBP antibody did not react with *F. solani*, *S. aureus*, *P. aeruginosa*, and HCE cells, whereas strong interaction of the PBP antibody was observed only with *A. castellanii* (Fig. 3C). These results demonstrated that the PBP antibody of *A. castellanii* specifically detected the *A. castellanii* trophozoites and cysts, which showed the potential for differential diagnosis for AK in mixed infection states.

To verify the cross-reactivity of the PBP antibody, ICC assay was performed using 5 different species of *Acanthamoeba* belonging to the morphological group II (*A. polyphaga* and *A. hatchetti*) and III (*A. culbertsoni*, *A. royreba*, and *A. healyi*). The trophozoites and cysts of the 5 *Acanthamoeba* species were detected by PBP antibody. However, *A. polyphaga* showed a weak reaction with the PBP antibody (Table 1).

Up to date, over 20 unique species of *Acanthamoeba* have been identified and 8 of them, which included *A. castellanii*, *A. polyphaga*, *A. royreba*, *A. cubertsoni*, *A. hatchetti*, *A. griffin*, *A. quiana*, and *A. lugdunensis* have been reported to cause keratitis [21]. In this study, the PBP peptide antibody detected trophozoites and cysts of 6 *Acanthamoeba* species associated with AK (Fig. 3; Table 1). Interestingly, the PBP antibody was able to detect *A. healyi* (Table 1) that can cause GAE [22]. These results showed that the PBP antibody could detect several species of *Acanthamoeba* trophozoites and cysts causing keratitis and GAE.

In conclusion, our study demonstrated the ability of the PBP polyclonal peptide antibody of *A. castellanii* to recognize various species of *Acanthamoeba*, as well as the potential for differential diagnosis of AK. Further validation of findings presented here using human clinical samples is warranted. The PBP antibody may enhance sensitivity of antibody-based diagnostic methods for *Acanthamoeba*-associated diseases.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest related to this study.

## REFERENCES

1. Marciano-Cabral F, Cabral G. *Acanthamoeba* spp. as agents of disease in humans. Clin Microbiol Rev 2003; 16: 273-307. https://doi.org/10.1128/CMR.16.2.273-307.2003
2. Schuster FL, Visvesvara GS. Free-living amoebae as opportunistic and non-opportunistic pathogens of humans and animals. Int J Parasitol 2004; 34: 1001-1027. https://doi.org/10.1016/j.ijpara.2004.06.004
3. Page MA, Mathers WD. *Acanthamoeba* keratitis: a 12-year experience covering a wide spectrum of presentations, diagnoses, and outcomes. J Ophthalmol 2013; 2013: 670242. https://doi.org/10.1155/2013/670242
4. Fanselow N, Sirajuddin N, Yin XT, Huang AJW, Stuart PM. *Acan-*

*thamoeba* keratitis, pathology, diagnosis and treatment. Pathogens 2021; 10: 323. https://doi.org/10.3390/pathogens10030323
5. Yera H, Ok V, Lee Koy Kuet F, Dahane N, Ariey F, Hasseine L, Delaunay P, Martiano D, Marty P, Bourges JL. PCR and culture for diagnosis of *Acanthamoeba* keratitis. Br J Ophthalmol 2021; 105: 1302-1306. http://dx.doi.org/10.1136/bjophthalmol-2020-316730
6. Rasheed AK, Siddiqui R, Ahmed SMK, Gabriel S, Jalal MZ, John A, Khan NA. hBN nanoparticle-assisted rapid thermal cycling for the detection of *Acanthamoeba*. Pathogens 2020; 9: 824. https://doi.org/10.3390/pathogens9100824
7. Maubon D, Dubosson M, Chiquet C, Yera H, Brenier-Pinchart MP, Cornet M, Savy O, Renard E, Pelloux H. A one-step multiplex PCR for *acanthamoeba* keratitis diagnosis and quality samples control. Invest Ophthalmol Vis Sci 2012; 53: 2866-2872. https://doi.org/10.1167/iovs.11-8587
8. Laummaunwai P, Ruangjirachuporn W, Boonmars T. A simple PCR condition for detection of a single cyst of *Acanthamoeba* species. Parasitol Res 2012; 110: 1569-1572. doi: 10.1007/s00436-011-2662-3
9. Turner ML, Cockerell EJ, Brereton HM, Badenoch PR, Tea M, Coster DJ, Williams KA. Antigens of selected *Acanthamoeba* species detected with monoclonal antibodies. Int J Parasitol 2005; 35: 981-990. https://doi.org/10.1016/j.ijpara.2005.03.015
10. Weber-Lima MM, Prado-Costa B, Becker-Finco A, Costa AO, Bililad P, Furst C, de Moura JF, Alvarenga LM. *Acanthamoeba* spp. monoclonal antibody against a CPA2 transporter: a promising molecular tool for acanthamoebiasis diagnosis and encystment study. Parasitology 2020; 147: 1678-1688. https://doi.org/10.1017/S0031182020001778
11. Kang AY, Park AY, Shin HJ, Khan NA, Maciver SK, Jung SY. Production of a monoclonal antibody against a mannose-binding protein of *Acanthamoeba* culbertsoni and its localization. Exp Parasitol 2018; 192: 19-24. https://doi.org/10.1016/j.exppara.2018.07.009
12. Park SM, Lee HA, Chu KB, Quan FS, Kim SJ, Moon EK. Production of a polyclonal antibody against inosine-uridine preferring nucleoside hydrolase of *Acanthamoeba castellanii* and its access to diagnosis of *Acanthamoeba* keratitis. PLoS One 2020; 15: e0239867. https://doi.org/10.1371/journal.pone.0239867
13. Lee HA, Chu KB, Kim MJ, Quan FS, Kong HH, Moon EK. Chorismate mutase peptide antibody enables specific detection of *Acanthamoeba*. PLoS One 2021; 16: e0250342. https://doi.org/10.1371/journal.pone.0250342
14. Singh A, Sahu SK, Sharma S, Das S. *Acanthamoeba* keratitis versus mixed *acanthamoeba* and bacterial keratitis: comparison of clinical and microbiological profiles. Cornea 2020; 39: 1112-1116. https://doi.org/10.1097/ICO.0000000000002337
15. Raghavan A, Baidwal S, Venkatapathy N, Rammohan R. The *Acanthamoeba*-fungal keratitis study. Am J Ophthalmol 2019; 201: 31-36. https://doi.org/10.1016/j.ajo.2019.01.024
16. Moon EK, Choi HS, Park SM, Kong HH, Quan FS. Comparison of proteins secreted into extracellular space of pathogenic and non-pathogenic *Acanthamoeba* castellanii. Korean J Parasitol 2018; 56: 553-558. https://doi.org/10.3347/kjp.2018.56.6.553
17. Ko W, Kim S, Lee HS. Engineering a periplasmic binding protein for amino acid sensors with improved binding properties. Org Biomol Chem 2017; 15: 8761-8769. https://doi.org/10.1039/C7OB02165H
18. Edwards KA, Baeumner AJ. Periplasmic binding protein-based detection of maltose using liposomes: a new class of biorecognition elements in competitive assays. Anal Chem 2013; 85: 2770-2778. https://doi.org/10.1021/ac303258n
19. Edwards KA, Randall EA, Tu-Maung N, Sannino DR, Feder S, Angert ER, Kraft CE. Periplasmic binding protein-based magnetic isolation and detection of thiamine in complex biological matrices. Talanta 2019; 205: 120168. https://doi.org/10.1016/j.talanta.2019.120168
20. Baek M, DiMaio F, Anishchenko I, Dauparas J, Ovchinnikov S, Lee GR, Wang J, Cong Q, Kinch LN, Schaeffer RD, Millán C, Park H, Adams C, Glassman CR, DeGiovanni A, Pereira JH, Rodrigues AV, van Dijk AA, Ebrecht AC, Opperman DJ, Sagmeister T, Buhlheller C, Pavkov-Keller T, Rathinaswamy MK, Dalwadi U, Yip CK, Burke JE, Garcia KC, Grishin NV, Adams PD, Read RJ, Baker D. Accurate prediction of protein structures and interactions using a three-track neural network. Science 2021; 373: 871-876. https://doi.org/10.1126/science.abj8754
21. Maycock NJ, Jayaswal R. Update on *Acanthamoeba* keratitis: diagnosis, treatment, and outcomes. Cornea 2016; 35: 713-720. https://doi.org/10.1097/ICO.0000000000000804
22. Moon EK, Xuan YH, Kong HH. Microarray and KOG analysis of *Acanthamoeba* healyi genes up-regulated by mouse-brain passage. Exp Parasitol 2014; 143: 69-73. https://doi.org/10.1016/j.exppara.2014.05.012