Indian Journal of Experimental Biology
Vol. 64, May 2026, pp, 394-399
DOI: 10.56042/ijeb.v64i05.24289

Effective method for studying β5 subunit activity of immunoproteasome *in vitro*

Guo Jinxing[1], Hu Jiahang[1] & Fu Dan[2]*

[1]HongQi Medicine, [2]College of Radiation and Imaging Science, Mudanjiang Medical University, Tongxiang Road 3, Mudanjiang 157011, China

Received 28 September 2025; revised 9 January 2026

The immunoproteasome and its dysfunction are implicated in multiple diseases. In multiple myeloma, immunoproteasomes promote cancer cell survival, making them an important therapeutic target for antagonist development. Here, we present a straightforward method for detecting β5i (the β5 subunit of the immunoproteasome) cellular activity *in vitro*. This cell-based approach utilizes a specific β5i substrate (Ac-Ala-Asn-Trp-AMC), which is cleaved by immunoproteasomes and releases a fluorescent signal with an emission peak at 460 nm. After multiple optimizations, we found that adding an equal volume of substrate solution to 30 µL cell lysate, incubating for 10 minutes at 37°C, and measuring fluorescence at 460 nm yielded IC_{50} values for ONX-0914 (a selective inhibitor of low-molecular mass polypeptide-7) and bortezomib that are consistent with published data, with repeatable and stable results across different cell lines. Additionally, comparison with the β5c commercial kit (Promega, G8661), which is compatible with the β5c substrate, demonstrated excellent sensitivity and accuracy. In summary, this protocol facilitates the screening and determination of subunit specificity for novel immunoproteasome inhibitors.

Keywords: Immunoproteasomes,Bortezomib, ONX-0914 (selective inhibitor of low-molecular mass polypeptide-7), β5i (β5 subunit of immunoproteasome)