**Description**

The IDE (Insulin-degrading enzyme) Inhibitor Screening Assay is a homogeneous fluorogenic assay designed to measure the activity of IDE for screening and profiling applications. The IDE assay kit comes in a convenient 384-well format and contains enough purified IDE (amino acids 71-254), substrate, and PR-02 buffer for 400 reactions.

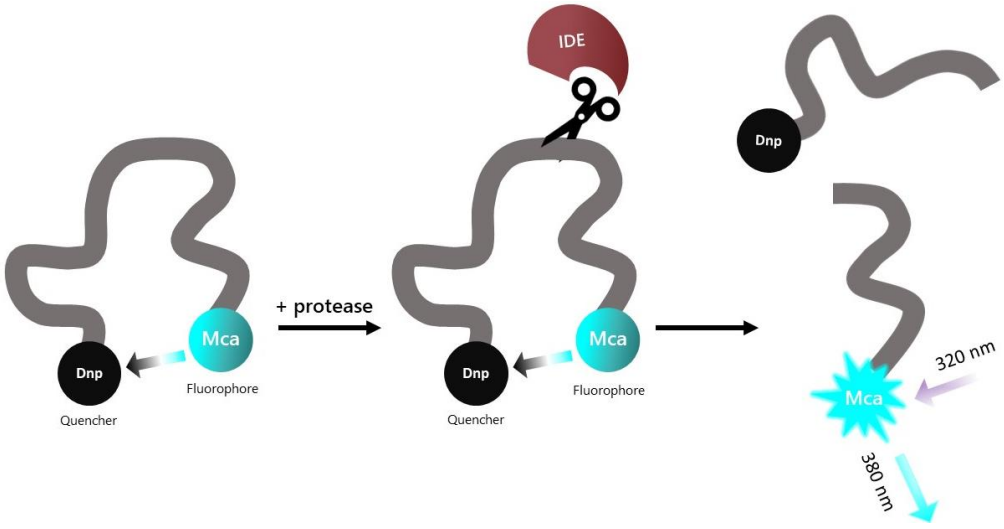


*Figure 1: Illustration of the assay principle.*
The substrate is an internally quenched fluorogenic substrate. Proteolysis releases the highly fluorescent Mca from the quencher. Fluorescence intensity increases proportionally to the activity of the protease.

**Background**

IDE (Insulin-degrading enzyme) is a zinc metallopeptidase with a ubiquitous pattern of expression in humans. Although initially identified as an insulin degrading protein, it can also interact with glucagon, somatostatin, β-amyloid and natriuretic peptide. IDE is involved in multiple pathways, such as Varicella Zoster Virus infection, steroid receptor signaling and proteasomal modulation of insulin. More recently IDE was identified as a heat-shock like protein capable of processing antigenic peptides presented by MHC class I. Dysregulation of IDE may be involved in Alzheimer's disease, Type 2 Diabetes Mellitus, and it may play a role in neuroblastoma growth. The identification of IDE inhibitors is crucial for the understanding of IDE role in disease and potential therapeutical approaches.

**Applications**

Screen small molecule inhibitors in high throughput screening (HTS) applications.

**Supplied Materials**

| Catalog # | Name | Amount | Storage |
|---|---|---|---|
| 70002 | IDE, His-Tag* | >2 µg | -80°C |
| | PR Substrate 1 | 2 x 5 µl | -80°C |
| | PR-02 Buffer | 2 x 10 ml | -20°C |
| 79961 | 384-well black microplate | 1 | Room Temp |

** The concentration of protein is lot-specific and will be indicated on the tube containing the protein.*



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**Stability**

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

**Safety**

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

**Assay Protocol**

- All samples and controls should be performed in duplicate.
- The assay should include "Negative Control", "Positive Control" and "Test inhibitor".
- If the assay plate is going to be used more than once, prepare enough reagents for this portion of the assay and aliquot the remaining undiluted reagents into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C or at -20°C as appropriate.

1. Thaw **IDE**, on ice. Briefly spin the tubes to recover the full content.

2. Dilute IDE to 0.5 ng/µl in **PR-02 Buffer** (10 µl/well).

   *Note: Keep the diluted protein on ice until use. Discard any unused diluted protein after use.*

3. Prepare the Test Inhibitor (2.5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 25 µl.

   a) If the Test Inhibitor is water-soluble, prepare serial dilutions 10-fold more concentrated than the desired final concentrations using the PR-02 Buffer. For the positive and negative controls, use PR-02 Buffer (Diluent Solution).

   b) If the Test inhibitor is soluble in DMSO, prepare in 100% DMSO at a concentration 100-fold higher than the highest desired concentration, then dilute the inhibitor 10-fold in PR-02 Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%. Use 10% DMSO in PR-02 Buffer (vol/vol) for the serial dilution to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in PR-02 Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   The final concentration of DMSO should not exceed 1%.

4. Add 10 µl of diluted IDE to all wells except the "Negative Control".

5. Add 10 µl of PR-02 Buffer to the "Negative Control" wells.

6. Add 2.5 µl of inhibitor solution to each well designated "Test Inhibitor".



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7. Add 2.5 µl of Diluent Solution to the "Positive Control" and "Negative Control" wells.

8. Preincubate the "Test inhibitor" with the diluted IDE for 30 minutes at Room Temperature (RT) with gentle agitation.

9. Dilute **PR Substrate 1** 2000-fold in PR-02 buffer.

10. Add 12.5 µl of the diluted PR Substrate to all wells. Protect your samples from direct exposure to light.

| **Component** | **Negative Control** | **Positive Control** | **Test Inhibitor** |
|---|---|---|---|
| PR-02 Buffer | 10 µl | - | - |
| Diluted IDE (0.25 ng/µl) | - | 10 µl | 10 µl |
| Test inhibitor | - | - | 2.5 µl |
| Diluent Solution | 2.5 µl | 2.5 µl | - |
| 30 minutes at Room Temperature | | | |
| Diluted PR Substrate 1 (2000-fold diluted) | 12.5 µl | 12.5 µl | 12.5 µl |
| **Total** | **25 µl** | **25 µl** | **25 µl** |

11. Incubate at Room Temperature for 30-60 minutes or perform kinetic analysis.

12. Read fluorescence intensity of the samples (lexcitation = 320 nm; lemission = 380 nm) in a fluorescence plate reader.



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**Example Results**

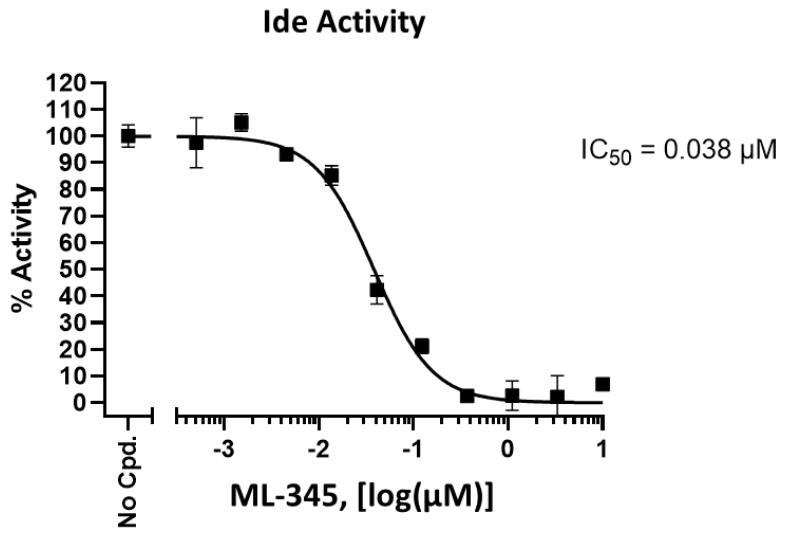


*Figure 2. IDE activity is inhibited by ML-345.*
IDE activity was measured in the presence of increasing concentrations of ML-345 (Cayman #17633). Results are expressed as percentage of activity relative to the positive control (measured in the absence of inhibitor and set at 100%).

*Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com*

**Troubleshooting Guide**
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

**Related Products**

| *Products* | *Catalog #* | *Size* |
|---|---|---|
| IDE, His-Tag recombinant | 70002 | 20 µg |
| Human Insulin Recombinant | 90202 | 50 mg/250 mg |
| Human Insulin Like Growth Factor-I Recombinant | 90166 | 20 µg/50 µg |


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