ab308154 – Human Insulin Fibrillation Inhibitor Screening Kit (Fluorometric)

For screening potential inhibitors of human insulin fibrillation
For research use only - not intended for diagnostic use.

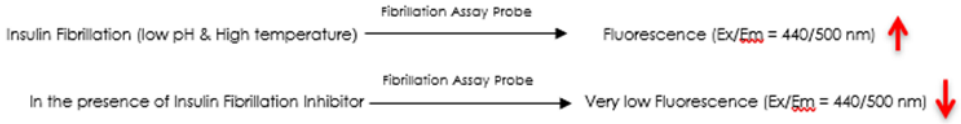
For overview, typical data and additional information please visit:

http://www.abcam.com/ab308154

Storage and Stability
The entire Assay kit may be stored at -20 °C and protected from light.

Introduction:

Human insulin consists of 51 amino acids, with a molecular mass of 5.81 kDa. It contains two peptide chains (A & B), which are linked by two disulfide bonds. Human insulin is widely used as a drug for treating diabetes patients. However, under certain conditions, human insulin aggregates into insoluble fibrils via fibrillogenesis, which leads to complications including reduced insulin potency, occlusion of insulin delivery devices, potentially increased immunological potential etc. Therefore screening of potential Inhibitors of human insulin fibrillation is critical for preventing insulin aggregation and developing potential therapeutics. In **The Human Insulin Fibrillation Inhibitor Screening kit,** insulin fibrillation is induced by low pH and high temperature. Fibrillation Assay Probe then binds to the beta sheets of the insoluble fibrils to generate a strong fluorescence signal measured at Ex/Em = 440/500 nm. But in the presence of an insulin fibrillation inhibitor, fibrillation is inhibited thereby reducing the fluorescence signal. The assay is simple, reliable and is high-throughput adaptable.



Materials Supplied

Item	Quantity
Fibrillation Assay Buffer	12 mL
Insulin Reconsitiution Buffer	15 mL
Neutralization Buffer	500 µl
Fibrillation Assay Buffer	20 µl
Human Insulin	5 vials
Insulin Fibrillation Inhibitor	20 µl

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Anhydrous DMSO
- Eppendorf thermomixer
- 1.5 ml microcentrifuge tubes with snap caps
- 96-well white plate with flat bottom
- Multi-well spectrophotometer (fluorescent plate reader)

Reagent Preparation

- Store the kit at -20 °C, protected from light.
- Briefly centrifuge all small vials prior to opening
- Read the entire protocol before performing the assay

Fibrillation Assay Buffer, Insulin Reconstitution Buffer, and Neutralization Buffer: Bring to room temperature (RT) before use. Store at 4 °C.

Fibrillation Assay Probe: Thaw the vial before use. Add 198 µl of anhydrous DMSO to the vial and mix by pipetting. Divide into aliquots and store at -20 °C. Avoid repeated freeze and thaw cycles. Use within two months.

Human Insulin: Reconstitute each vial with 1.1 ml of Insulin Reconstitution Buffer. Mix well by inverting several times. Keep on ice while in use. Divide into aliquots and store at -20 °C. Avoid repeated freeze and thaw cycles. Use within two months.

Insulin Fibrillation Inhibitor: Thaw the vial at RT before use. Avoid repeated freeze-thaw cycles. Divide into aliquots and store at -20 °C. Use within two months.

Sample Preparation

Human Insulin Preparation: Prepare enough Human Insulin for the number of assays to be performed. Add 50 µl of reconstituted Human Insulin into three microcentrifuge tubes labeled as Inhibitor Screen (IS), Human Insulin Fibrillation Control (PC) and Inhibitor Control (IC) respectively.

Screening Compounds, Inhibitor Control, Human Insulin Fibrillation Control, and Blank Control Preparation: Dissolve candidate inhibitors into an appropriate solvent at 50X concentration to be tested. Prepare a 50 fold dilution of the Inhibitor Screen compounds using Insulin Reconstitution Buffer and add 50 µl of diluted candidate inhibitor(s) to the Inhibitor Screen (IS) tube(s) from step 1. Add 50 µl of Insulin Reconstitution Buffer to the Insulin Fibrillation Control (PC) tube from step 1. Prepare a 50 fold dilution of the Insulin Fibrillation Inhibitor (e.g. 5 µl of Insulin Fibrillation Inhibitor into 245 µl of Insulin Reconstitution Buffer) and mix well. Add 50 µl of the diluted inhibitor into the Inhibitor Control (IC) tube. For Blank Control (BC), add 100 µl of Insulin Reconstitution Buffer into the Blank Control tube. The volume of all tubes including IS, PC, IC and BC in this step is 100 µl. Place all the tubes in a 60 °C thermomixer, and incubate at 300 rpm for 24 hr.
Note: If the Solvent(s) is expected to affect the Insulin Fibrillation process, prepare a parallel well(s) for the Solvent Control (SC) to test the effect of the solvent on the insulin fibrillation process. To the SC tube, add 50 µl of the final solvent concentration and 50 µl of human insulin.

In case SC value is significantly different from Insulin Fibrillation Positive Control, use its value to determine the effect of candidate compound(s).

Assay Procedure

1. **Reaction Mix Preparation**: Prepare enough reagents for the number of assays to be performed. For each well, prepare 100 µl of Reaction Mix containing:

	Reaction Mix
Insulin Fibrillation Assay Buffer	98 µl
Fibrillation Assay Probe	2 µl

   Mix and add 100 µl of Reaction Mix into each well including IS, PC, IC and BC. Cover the plate with an aluminum foil and gently shake for 5 min and incubate for another 15 min at RT.
2. Measurement: Measure the fluorescence at Ex/Em = 440/500 nm.
3. Calculation: Set the RFU of Human Insulin Fibrillation Control (PC) as the 100% and calculate the % relative activity of Candidate Inhibitors as following.

$$\text{\% Relative Activity} = \left(\frac{\text{RFU of Candidate Inhibitors}}{\text{RFU of Human Insulin Fibrillation Control}}\right) \times 100\%$$

Note: If the Solvent Control (SC) values are significantly different from the Insulin PC, use these values in the equation above, after subtracting the Blank Control (BC).

For technical support contact information, visit: www.abcam.com/contactus