

InsulinQC™

Pre-fibrillar insulin species in biopharma production

InsulinQC™

BioChromix's InsulinQC™ is a unique kit for assaying insulin aggregates in biopharmaceutical production. The kit is based on BioChromix's BCX500 probe which specifically targets pre-fibrillar and to some extent fibrillar insulin species. Gel and fibril formation is a commonly occurring problem in insulin biopharma production, resulting in low batch quality. The high specificity and sensitivity of InsulinQC™-BCX500 offers the possibility to detect ultra-low levels of pre-fibrillar species and thereby quantifying unwanted species of the insulin batch at a very early stage. Other insulin samples can be assayed as well. The Insulin-QC kit is superior to conventional fibrils detection probes such as Thioflavine T (ThT). This novel and superior method is based on BioChromix's patented optical Apolyon™ probes.

How it works

The BCX500 probe is highly specific towards pre-fibrillar, and fibrillar to some extent, insulin species which is evaluated by means of fluorescence spectroscopy. The interaction between BCX500 and pre-fibrillar insulin species results in characteristic shift in fluorescence emission wavelength as well as a major fluorescence intensity increase. The kit procedure is very simple and robust. The insulin sample is resolved or diluted in an assay buffer, whereupon the BCX500 probe is added. After a few minutes incubation the fluorescence of the sample can be read, either in a plate or cuvette format. The assay is easily evaluated either by measuring the fluorescence intensity $I_{\text{Ins_QC}} = I_{516 \text{ nm}}$ or by the fluorescence ratio $R_{\text{Ins_QC}} = I_{516 \text{ nm}}/I_{620 \text{ nm}}$. Below an example of bovine insulin fibrillation kinetics, using BioChromix InsulinQC™ in comparison with ThT is illustrated. The early response that BioChromix InsulinQC™ provides clearly manifests the specificity for pre-fibrillar species and demonstrates the advantage compared to a conventional method such as ThT.

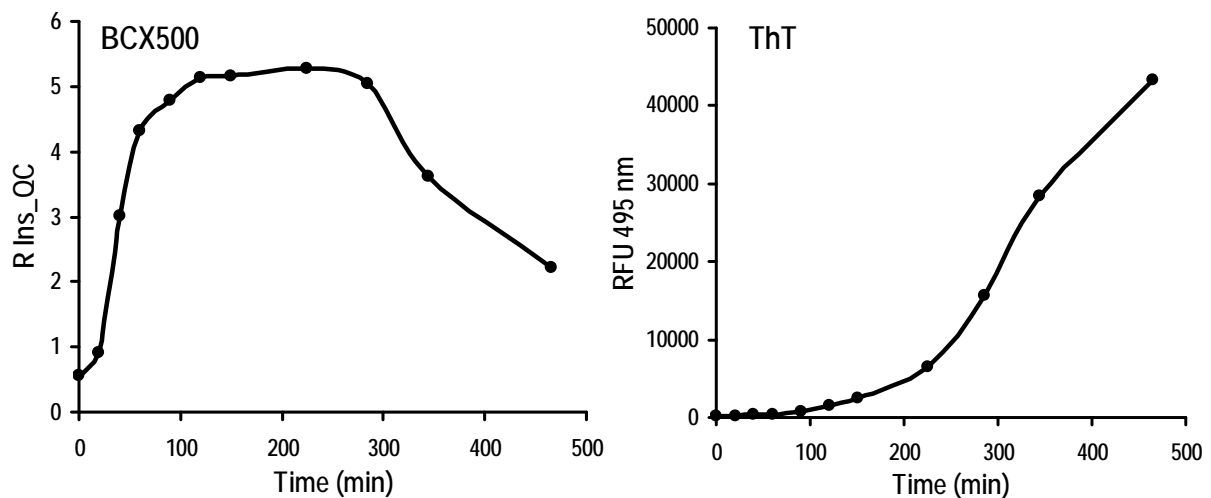


Figure 1. Insulin fibrillation kinetics, 0 min only native insulin present and increasing number and size of insulin aggregates with time. Left) Insulin fibrillation kinetics monitored with BCX500. A strong signal increase in the fluorescence ratio $R_{\text{Ins_QC}}$ is obvious already after a little bit more than 20 min. Right) Insulin fibrillation kinetics monitored with ThT. A signal increase can be seen after 100-200 min, but a strong signal increase is only evident after more than 200 min.

What it's used for

BioChromix's InsulinQC™ can be used to detect and quantify pre-fibrillar species in insulin in biopharma production. It is also possible to use the kit for other insulin containing samples as well. The InsulinQC™ is available for purchase in 10000 or 20000 units which is enough for 5000-10000 samples or 10000-40000 samples, respectively, depending on the measurement format.

Advantages of BioChromix InsulinQC™ kit

With InsulinQC™ the quality control of insulin is fast, easy, robust and does normally not require heat incubation of the insulin sample. The BCX500 is a unique probe that detects very early pre-fibrillar states indicative of low insulin batch quality. Some of the advantages can be summarized in the following sentences:

- Specific to pre-fibrillar insulin species
- Few-step, simple assay procedure
- Assay read-out with conventional fluorescence reader
- Ratiometric read-out possible, no standards needed.

Requirements

Equipment: Fluorescence plate reader or fluorescence spectrometer with excitation at $\sim 450 \pm 7.5$ nm and emission at $\sim 516 \pm 7.5$ nm and $\sim 620 \pm 7.5$ nm (recommendations only).

Material: Buffer solutions for sample dilution. InsulinQC™ can be used up to pH 10.

References

Representative scientific publications are listed below: Paper 1 deals with detection of amyloid fibers in solution at acidic pH using conjugated polyelectrolytes, and paper 2 more specifically with Insulin fibrillation kinetics and the elongation pathway of insulin amyloid fibrils.

1. Anna Herland, K. Peter R. Nilsson, Johan D. M. Olsson, Per Hammarström, Peter Konradsson, and Olle Inganäs. *Synthesis of a Regioregular Zwitterionic Conjugated Oligoelectrolyte, Usable as an Optical Probe for Detection of Amyloid Fibril Formation at Acidic pH*, J Am Chem Soc (2005), **127**,2317-2323.
2. Bente Vestergaard, Minna Groenning, Manfred Roessle, Jette S. Kastrop, Marco van de Weert, James M. Flink, Sven Frokjaer, Michael Gajhede, and Dmitri I. Svergun. *A Helical Structural Nucleus Is the Primary Elongating Unit of Insulin Amyloid Fibrils*, PLoS Biology (2007), **5(5)**,1089-1097.

Patent pending: InsulinQC™ is protected by US 11/579,741 including corresponding applications and additional patent pending by BioChromix.

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