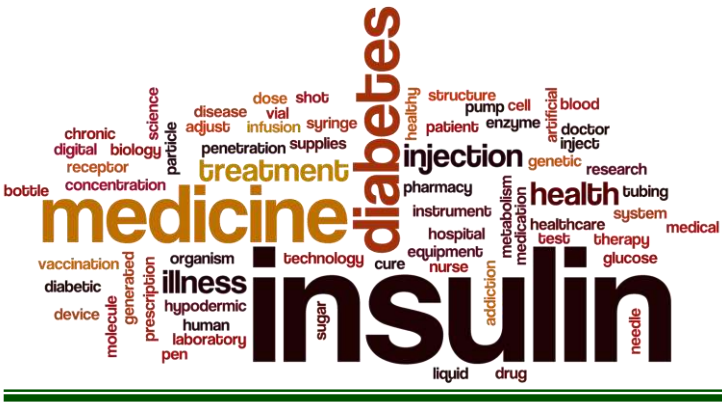


Demonstrating Biosimilarity

Proteomics International (PI)

Founded in 2001, PI is a specialist testing facility operating in Australia under the Asian time zone.

As the world's first company to receive ISO 17025 laboratory accreditation for proteomics services, PI provides specialist analytical testing services for the pharmaceutical and biotechnology industries.

PI will map a biosimilar product to determine whether there is a fingerprint-like similarity profile compared to the reference product, in accordance to **FDA**, **EMA** and **ICH Q6B** test procedures and guidelines.

Expertise in Biosimilars Analysis

Biosimilars are complex. Minor changes during the manufacturing process can alter the protein's efficacy and safety.

To gain regulatory approval, the FDA requires biosimilars manufacturers to demonstrate "the molecular weight of the protein, its higher order structure and post-translational modifications, heterogeneity, functional properties, impurity profiles, and degradation profiles denoting stability".

PI provides accredited biosimilars testing services that ensures a biosimilar product meets the FDA's stringent physicochemical and structural requirements for regulatory approval.

INSULIN

- Two polypeptide chains (A and B chains)
- Linked by 2 disulphide bridges and an additional bridge within the A chain
- Human insulin and its three analogues – differ by 1-3 residues

Type	Generic name	Sequence
Rapid-acting	❑ **Insulin aspart**	GIVEQCCTSICSLYQLENYC**N** FVNQHLCGSHLVEALYLVCGERGFFYT**DK**T
	❑ **Insulin lispro**	GIVEQCCTSICSLYQLENYC**N** FVNQHLCGSHLVEALYLVCGERGFFYT**KP**T
Short-acting	❑ **Human insulin**	GIVEQCCTSICSLYQLENYC**N** FVNQHLCGSHLVEALYLVCGERGFFYT**PK**T
Long-acting	❑ **Insulin glargine**	GIVEQCCTSICSLYQLENYC**G** FVNQHLCGSHLVEALYLVCGERGFFYT**PK**T**RR**

Quality, Identity and Purity

Structural characterisation & confirmation	Physicochemical properties
Peptide mapping analysis	Intact mass analysis by LCMS
Disulphide bridge analysis	Impurity profile and characterisation
N/C terminal sequencing by MS	CD analysis
N-terminal sequencing	Aggregation analysis
Amino acid analysis	Fluorescence spectrometry

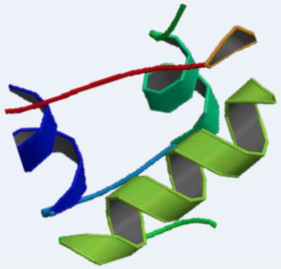
Proteomics International

A trusted partner for demonstrating biosimilarity for global regulatory approvals

USA | Japan | Singapore | Brazil | India | China | Hong Kong | Malaysia | Australia

info@proteomics.com.au
www.proteomics.com.au

Human Insulin



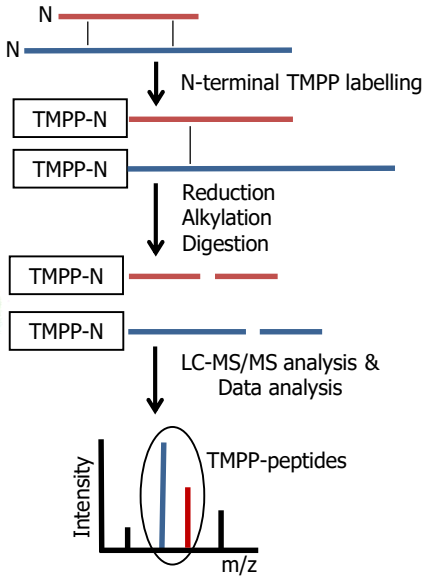
Human Insulin Sequence

GIVEQCCTSICSLYQLENYCN

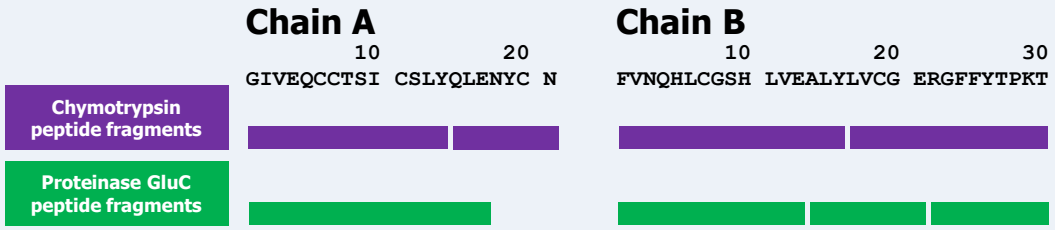
FVNQHLCGSHLVEALYLVCGERGFFYTPKT

N-terminal sequence analysis by mass spectrometry

This analysis utilises N-terminal protein labelling with *N*-succinimidyloxycarbonylmethyl) tris (2,4,6-trimethoxyphenyl) phosphnium bromide (TMPP).
Labelled insulin is reduced, alkylated, digested and analysed through LC-MS/MS. MS/MS spectra of N-terminal peptides are analysed by the sequence matching software Mascot [Matrix Science].



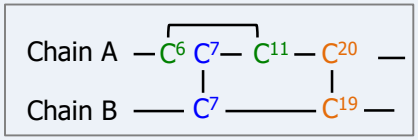
Peptide mapping analysis confirms the complete primary sequence of the molecule by mass spectrometry, using a multiple enzyme digest strategy. Data analysis incorporates the latest algorithms and includes options for *de novo* sequencing.



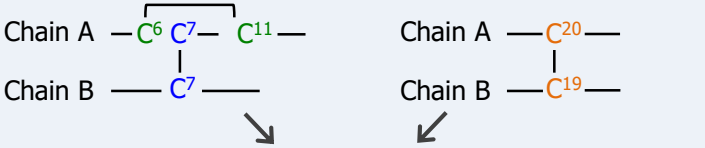
Demonstrating protein functionality

The biosimilar production process can lead to incorrect disulphide bond pairings. This will result in a loss of functionality and efficacy.
PI's **disulphide bridge analysis** will demonstrate whether the insulin molecule is correctly folded and ready for functional characterisation.
By mapping the position of each disulphide bond through different reduction and alkylation conditions, PI's LC-MS/MS analysis eliminates downstream functional characterisation issues by confirming that the molecule is correctly folded.

Disulphide bridge analysis experimental design



Step 1: Enzyme digestion



Step 2: Targeted treatment with reducing & Alkylating agent 1

Step 3: Complete treatment with reducing & Alkylating agent 2