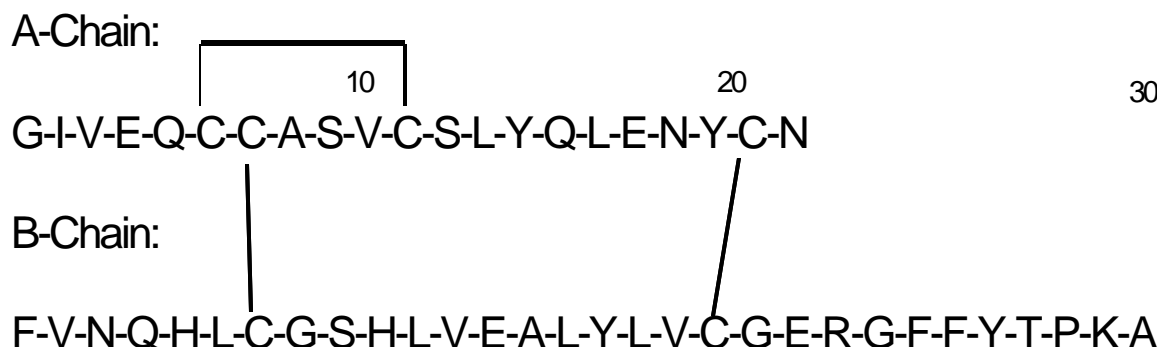


## INSULIN from Bovine Pancreas

Sigma Prod. No. **I5500, I6634, I1882, and I4011**

**CAS NUMBER:** 11070-73-8

### STRUCTURE:<sup>1</sup>



Interchain disulfide bonds are located between the cysteines at positions A7 and B7 and between positions A20 and B19. An intrachain disulfide bond occurs between the cysteines at A6 and A11.<sup>1,2</sup> Bovine insulin differs from human insulin at the following positions: alanine for threonine at A8, valine for isoleucine at A10, and alanine for threonine at the carboxyl terminal of the B-chain.<sup>1</sup>

### PHYSICAL PROPERTIES:

Formula weight: 5733.5

Molecular formula: C<sub>254</sub>H<sub>377</sub>N<sub>65</sub>O<sub>75</sub>S<sub>6</sub>

Isoelectric point: pI = 5.3 for the native protein<sup>3</sup>

Sedimentation coefficient ( $S_{20,w}$ )<sup>4</sup>:  $1.95 \times 10^{-13}$

Diffusion coefficient ( $D_{20,w}$ )<sup>4</sup>:  $7.30 \times 10^{-7}$

Partial specific volume ( $V_{20}$ )<sup>4</sup>: 0.735 ml/g

Stokes radius (calculated): 11.9 Å

$E^{278\text{nm}}$  = 6080 (33 mM phosphate, pH 7.0)<sup>5</sup>

$E^{1\%}$ (278nm) = 10.6 (33 mM phosphate, pH 7.0)

Potency is determined by HPLC.<sup>2</sup>

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**METHOD OF PREPARATION:**

Insulin is purified from an ethanol/phosphoric acid extract of bovine pancreas by multiple ammonium sulfate and isoelectric precipitations. It is then crystallized without zinc followed by crystallization with zinc. The zinc content of the final product is approximately 0.5%. Zinc can be removed by solubilizing the insulin in dilute acetic acid, adding excess EDTA to chelate the zinc, and then precipitating the insulin at its pI.<sup>7</sup>

**STABILITY / STORAGE STABILITY:**

Insulin should be stored protected from light in a desiccator at -20°C.

**SOLUBILITY / SOLUTION STABILITY:**

Insulin has low solubility at neutral pH. It can be solubilized at 2 mg/ml in dilute acetic or hydrochloric acid, pH 2-3. A stock solution can be stored frozen at -20°C in single-use aliquots. Freeze-thaw cycles should be avoided. Alternatively, it can be stored for up to 12 months at 2-8°C if it is sterile filtered through a low protein binding membrane or if it contains a suitable bacteriostat, such as 0.1% thimerosal or sodium azide. Insulin solutions cannot be autoclaved. Insulin can also be solubilized in 125 mM NaHCO<sub>3</sub>.<sup>6</sup> However, alkaline stock solutions are not recommended since high pH increases the rate of deamidation and aggregation.

**GENERAL REMARKS:**

Insulin is produced *in vivo* in the pancreatic  $\beta$ -cells. The precursor protein (preproinsulin) contains a 23-30 amino acid signal peptide attached to the amino terminal of proinsulin. Proinsulin is composed of the insulin B-chain followed by a connecting peptide (C-peptide) and the A-chain. The signal peptide assists in translocating preproinsulin into the lumen of the endoplasmic reticulum, after which it is rapidly cleaved. Proinsulin is then transported to the Golgi complex where it is packaged into granules and converted to insulin. On secretion, equimolar amounts of insulin and C-peptide are released into the blood.<sup>8,9,10</sup>

Insulin is the primary hormone responsible for controlling the cellular uptake, utilization and storage of glucose, amino acids, and fatty acids while inhibiting the breakdown of glycogen, protein, and fat. Several excellent reviews of the biochemistry, physiology, and pharmacology of insulin have been published.<sup>9,10,11,12</sup> The insulin receptor is a tyrosine kinase that phosphorylates 185 kDa insulin receptor substrate (IRS-1) found in most cell types. IRS-1 activates phosphatidylinositol 3 kinase (IP<sub>3</sub> Kinase).<sup>13,14,15</sup> The K<sub>d</sub> for insulin at its receptor is approximately 0.5 nM (approximately 2.9 ng/ml).<sup>15</sup>

Bovine insulin is often included as a medium supplement for cell culture. The concentration range is 1-10  $\mu$ g/ml depending on the cell type.<sup>16,17</sup>

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