# Product Data Sheet

No. 1

## I27O™ AFM Reference Protein

Catalog Number: 0304


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### Product Description

I27O™ is a 94 kDa synthetic polyprotein composed of eight repeats of the I27 domain of human titin. It is used for the calibration of and as a reference for force extension experiments using atomic force microscopes. It is supplied in a ready-to-use solution. Single molecule measurements give up to eight saw-tooth force-extension curves with the specifications below. The protein has durable elasticity that allows for repeated unfolding and refolding.

### Product Specifications

| | |
|---|---|
| **Protein** | 100µg |
| **Shipping** | ships ambient or on wet ice |
| **Long-term Storage** | 10 years at -80°C<br>Aliquot to avoid repeated freezing and thawing |
| **Short-tem Storage** | 1 month at 4°C |
| **Formulation** | In SSI Buffer:<br>(50 mM $Na_2HPO_4$, 300 mM NaCl, 20 mM imidazole, pH 8.0) |

### Technical Information (force extension measurements)

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|---|---|
| **Distance between peaks** | 24.1 ± 0.34nm |
| **Force peaks** | 204 ± 26pN |
| **Persistence length** | 0.30 ± 0.07nm |
| **Countour length (per domain)** | 24.1 ± 0.34nm |

**Force-extension Relationships for Recombinant Poly-I27 Measured with AFM Techniques**

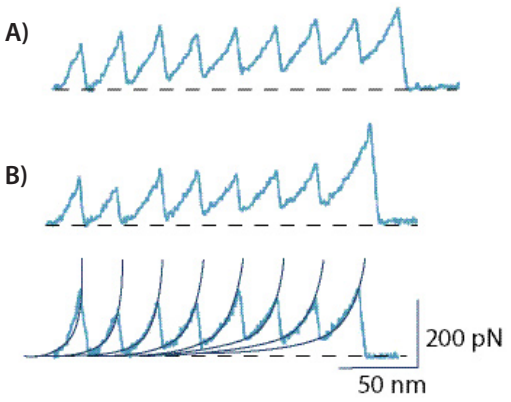


**Figure 1.** A) Sawtooth pattern of unfolding observed with the I27LG11 polyprotein. (Ref. 5) B) Stretching of single I27O™ polyproteins gave force-extension curves with a sawtooth pattern with equally spaced force peaks. The sawtooth pattern is well described by the WLC equation (B, bottom trace, continuous lines).

***See full technical information at www.athenaes.com***

### Instructions for Use (force extension measurements)

1. Dilute the stock protein solution to a concentration of 100µg/ml in PBS pH 7.3 buffer and apply the sample to freshly evaporated gold coverslips.

2. Attach coverslips to the piezoelectric positioner stage.

The I27O™ was engineered with an amino-terminal hex-His peptide sequence and carboxy-terminal di-Cys. This configuration is hypothesized to allow the protein molecules to attach to the coverslip at the carboxy-terminal leaving the amino-terminus free to attach to the silicon nitride cantilever tip, when the tip is brought in close proximity to the absorbed protein. It is this model which is believed to account for the high frequency (>50%) of observing all eight domain extensions after each retraction as well as the ability to perform extension and retraction experiments on a single molecule.

### Material Safety Data

FOR RESEARCH USE ONLY. NOT INTENDED OR APPROVED FOR HUMAN, DIAGNOSTICS OR VETERINARY USE. Do not ingest, swallow or inhale. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling. For complete safety information see full Material Safety Data Sheet.

### References


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2. Czajkowsky, D. M. and Shao, Z. 1998. Submolecular resolution of single macromolecules with atomic force microscopy. FEBS Lett. 430:51-54.
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4. Fisher, T. E., Oberhauser, A. F., Carrion-Vazques, M., Marszalek, P. E., and Fernandez, J. M. 1999. The study of protein mechanics with the atomic force microscope. TIBS 24:379-384.
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6. Marko, J. F. and Siggia, E. D. 1995. Stretching DNA. Macromolecules 28:8759-8770.
7. Oberhauser, A. F., Marszalek, P. E., Carrion-Vazques, M. and Fernandez, J. M. 1999. Single protein misfolding events captured by atomic force microscopy. Nature Struc. Biol. 6(11):1025-1028.
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