

Poly(I:C)-LMW

Validation Sheet

INTRODUCTION

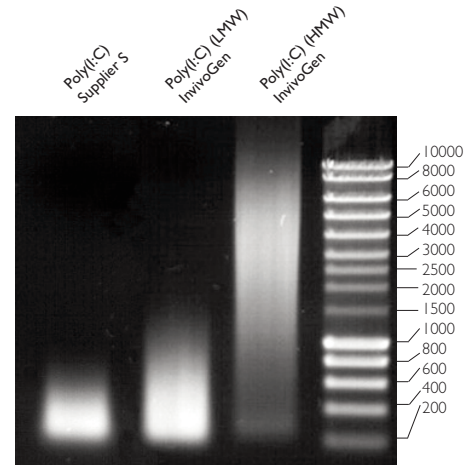
InvivoGen introduces a new preparation of poly(I:C), called poly(I:C) (LMW) in regards to its lower molecular weight. Its average size is 0.2-1 kb, whereas the average size of our other poly(I:C) preparation (catalog # tlr1-pic) is 1.5-8 kb. Both preparations are cell culture and endotoxin tested. The characteristics and activity of poly(I:C)s provided by InvivoGen and another supplier are listed below.

PRODUCT INFORMATION

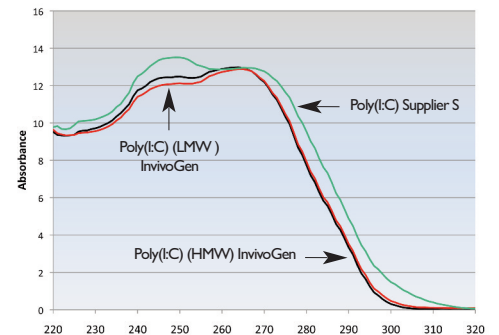
Name : Poly(I:C) (LMW)
Description : Synthetic analog of double-stranded RNA
Catalog codes : tlr1-picw (25 mg)
 tlr1 picw-250 (250 mg)

CHARACTERISTICS

Product (Supplier)	Poly(I:C) (LMW) (InvivoGen)	Poly(I:C) (HMW) (InvivoGen)	Poly(I:C) (Supplier S)
Size	0.2-1 kb	1.5-8 kb	0.2-0.6 kb
Absorbance ratio	A250/A260 : 0.95 A280/A260 : 0.62 A290/A260 : 0.28	A250/A260 : 0.97 A280/A260 : 0.60 A290/A260 : 0.26	A250/A260 : 1.05 A280/A260 : 0.73 A290/A260 : 0.38
Lambda max	266	264	249
Endotoxin levels	0.125-1.25 EU/mg	<0.125 EU/mg	125-1250 EU/mg
Solubility	20 mg/ml	1 mg/ml	10 mg/ml
<i>In vitro</i> working concentration	30 ng - 10 µg/ml	30 ng - 10 µg/ml	100 ng - 10 µg/ml

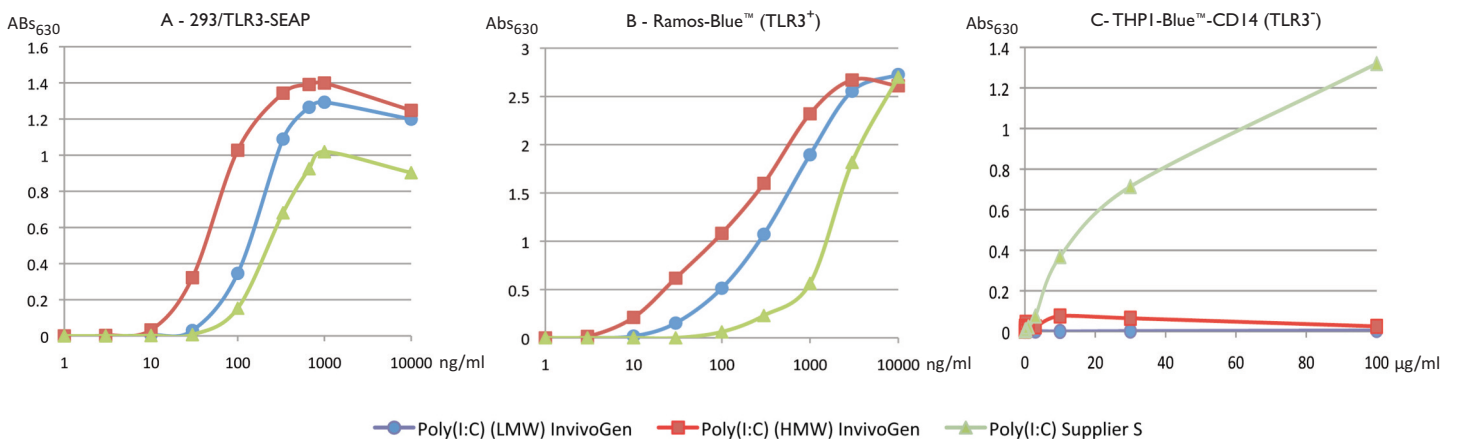


Poly(I:C)s were loaded on an agarose gel with a basepair ladder to assess their size. After electrophoresis, the gel was stained with ethidium bromide.



The UV/visible absorption spectrum was performed using poly(I:C) solutions at 1 mg/ml in NaCl 0.9% from λ 220 nm to λ 320 nm.

ACTIVITY



Poly(I:C) activity was determined in 293 cells stably expressing TLR3 (A), Ramos-Blue™ cells, a human B lymphocyte cell line (B) that expresses TLR3 endogenously, and THPI-Blue™-CD14 cells that do not express TLR3 (C). All three cells are stably transfected with an NF-κB-inducible SEAP reporter gene. The cells were stimulated with increasing concentrations of poly(I:C). After 24h incubation, NF-κB activation was assessed by measuring the levels of SEAP in the supernatant by using QUANTI-Blue™, a SEAP detection medium.