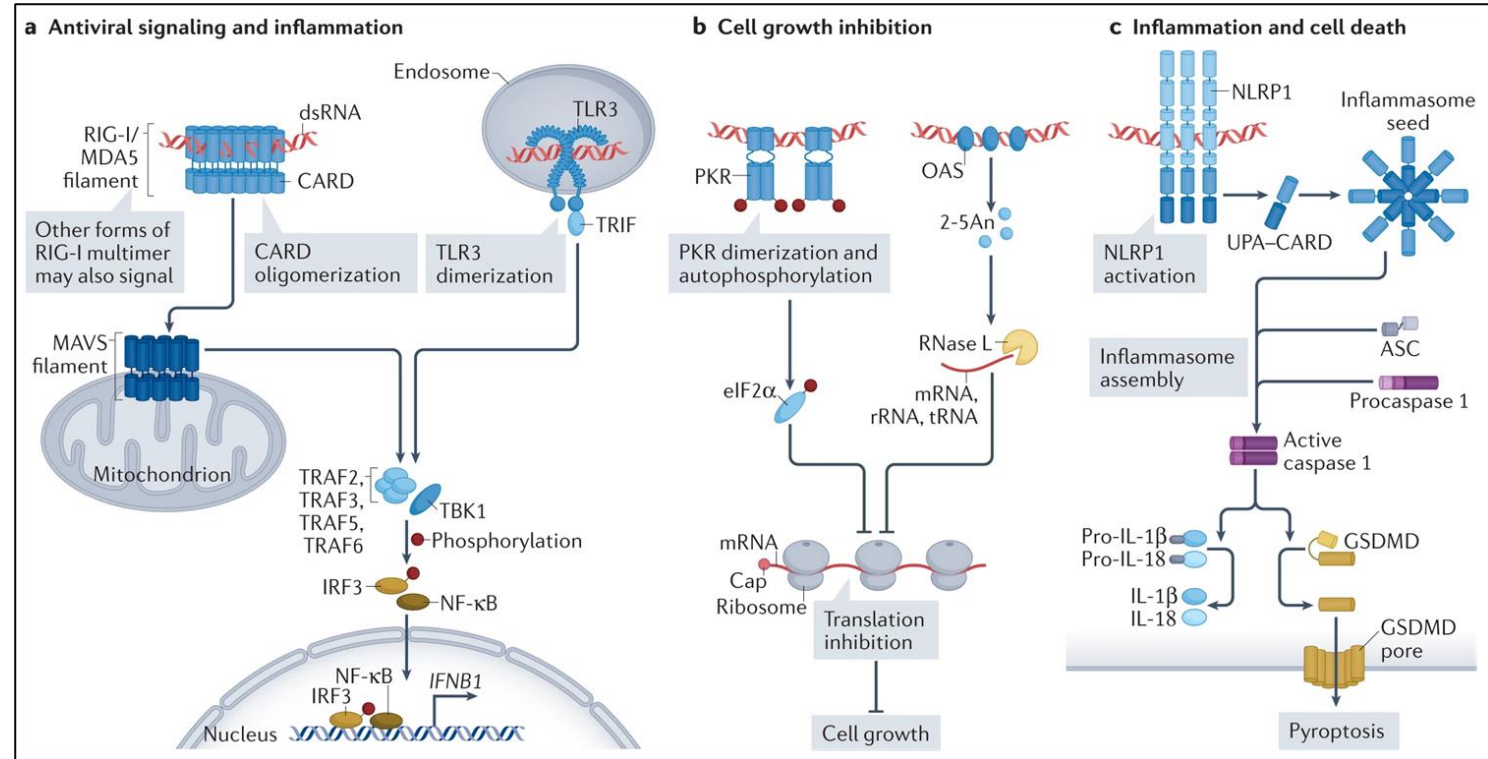


dsRNA is a contaminant in IVT drug substance

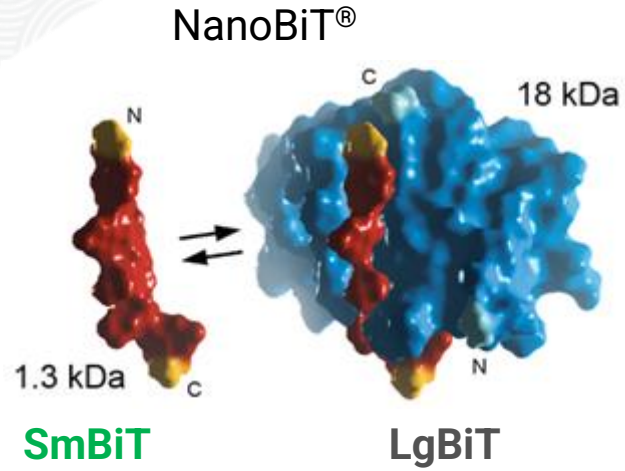
- dsRNA is a byproduct of in vitro transcription (IVT) products
- dsRNA is highly immunogenic and can be detected by several intracellular or endosomal sensors



- Existing methods to detect dsRNA in mixed solutions lack quantitation and sensitivity
- **We have developed a novel assay system for dsRNA detection using bioluminescence**

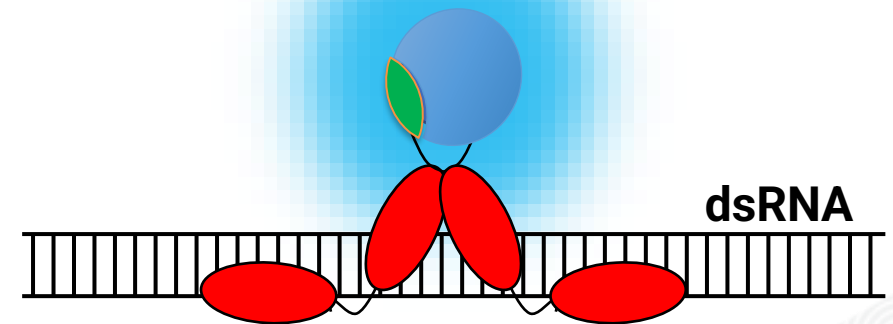
Lumit® dsRNA Detection Assay

dsRNA detection using Nanoluciferase Binary Technology (NanoBiT[®])

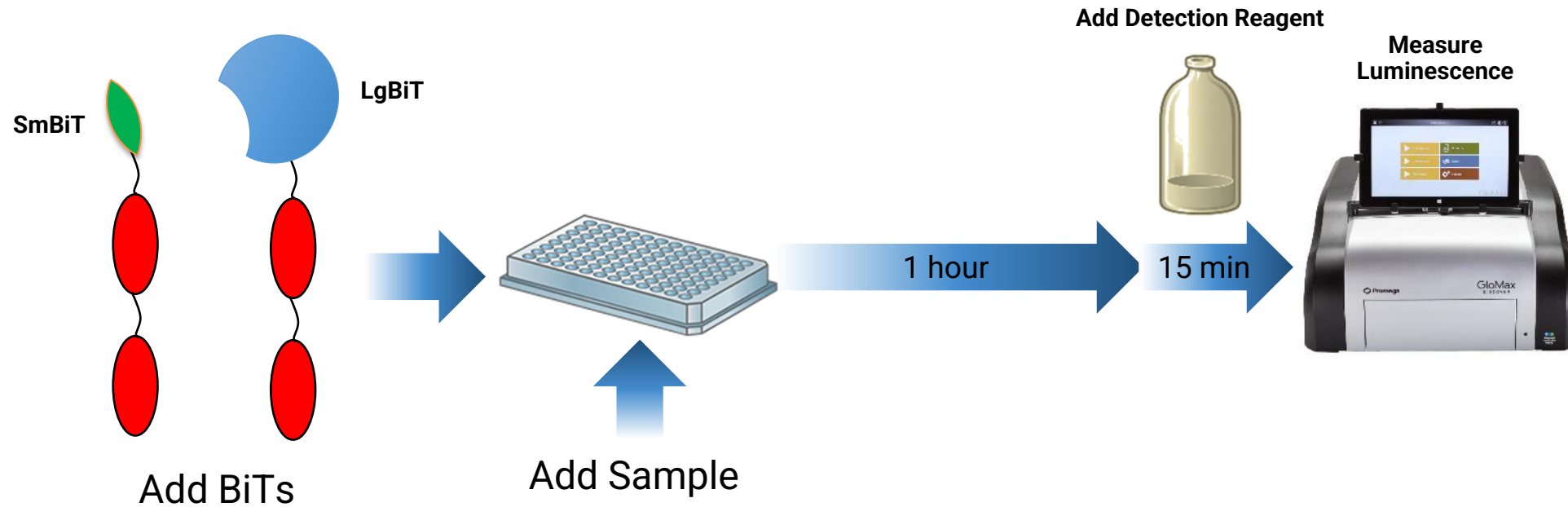


- Nanoluciferase is split into two subunits: SmBiT and LgBiT
- Complementation of SmBiT and LgBiT generates a functional luciferase and the generation of light in the presence of substrate
- SmBiT and LgBiT have very low affinity in solution

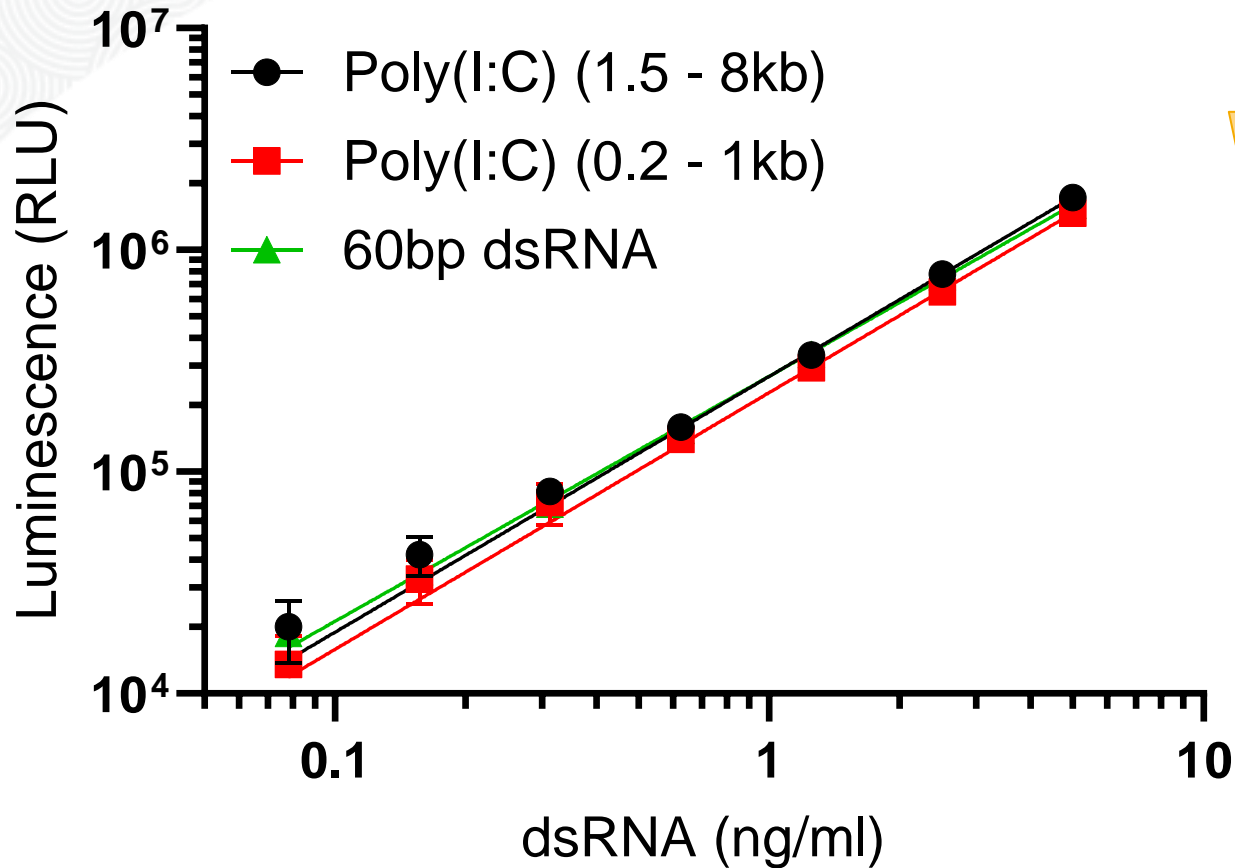
- Forced complementation of the NanoBiT[®] luciferase is achieved by fusion of the BiTs to dsRNA binding domains (red)
- When used to detect analytes, this technology is called **Lumit[™]**



Lumit™ dsRNA Detection Assay Protocol



dsRNA detection independent of sequence and size



Size

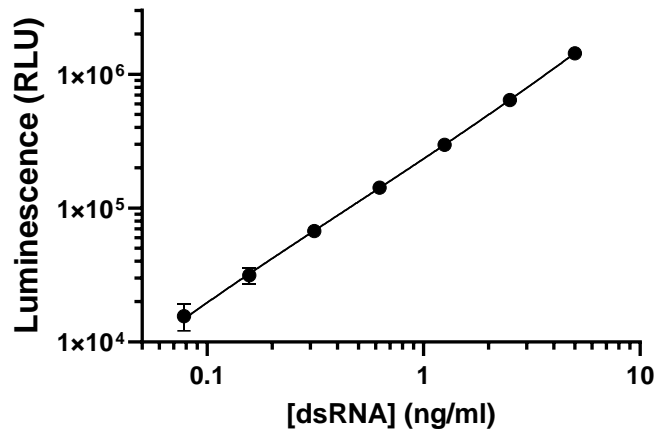


Sample	Slope	R ²
Poly(I:C) (1.5 – 8 kb)	1.15	0.997
Poly(I:C) (0.2 – 1 kb)	1.16	0.997
60bp dsRNA	1.11	0.999

- dsRNA samples ranging from 60 – 8000 bp were analyzed with the Lumit[®] dsRNA Detection Assay
- Samples were detected with similar slope and sensitivity
- **Assay can detect dsRNA ≥ 30 bp (not shown)**
- **Assay is highly specific for dsRNA (not shown)**

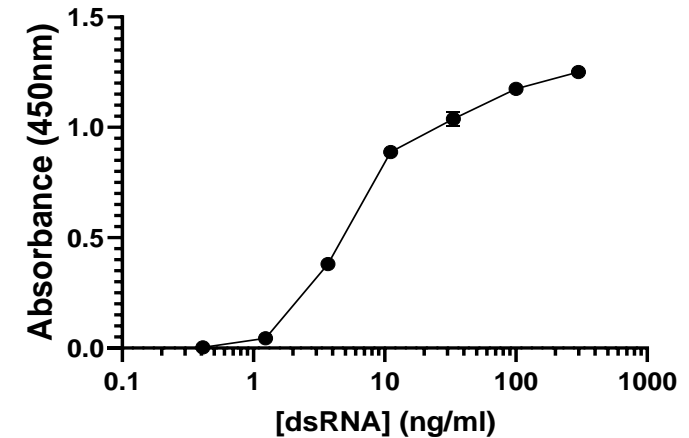
Benchmarking with commercial ELISA

Lumit[®] dsRNA Detection Assay



Sample	Expected	Observed
60bp dsRNA	0.16 ng/ml	0.15 ng/ml
60bp dsRNA	1.3 ng/ml	1.3 ng/ml
142bp dsRNA "standard"	1.6 ng/ml	0.91 ng/ml
Poly(I:C)	2.5 ng/ml	1.9 ng/ml
IVT Product	-	1.8 ng/ml

Commercial ELISA Kit



Sample	Expected	Observed
60bp dsRNA	5 ng/ml	Not detected
60bp dsRNA	75 ng/ml	Not detected
142bp dsRNA "standard"	20 ng/ml	2.2 ng/ml
Poly(I:C)	5 ng/ml	6.1 ng/ml
IVT Product	-	0.3 ng/ml

- Commercial dsRNA ELISA does not accurately quantitate native dsRNA
- It is likely that commercial dsRNA ELISAs significantly underestimate the amount of dsRNA in IVT products