

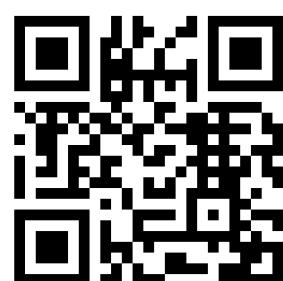
azooka

tinto rang™

THE WORLD'S SAFEST NUCLEIC ACID STAIN



FACTS SHEET



ISO 13485 CERTIFIED

RE-THINKING BIOLOGY WITH SAFETY, SUSTAINABILITY & PERFORMANCE

Go **GREEN** without compromise

Green chemistry is an approach that aims to maximize efficiency and minimize or eliminate the use and generation of hazardous substances that affect human health and the environment. Azooka is propelling the **GREEN** chemistry transition by re-inventing molecular biology with safety, sustainability, and performance in mind. Azooka Eco-Fluros are food-grade safe and adhere to all 12 principles of green chemistry while offering superior performance at an affordable price.

Key **GREEN** chemistry principles of Azooka Fluros



Designing Safer Chemicals



Biohazard Waste Prevention



Real-Time Pollution Protection



Less Hazardous Chemical Synthesis



Safer Chemistry For Accident Prevention



PRODUCT DESCRIPTION

tinto rang™ is a hypersensitive eco-fluorophore for visualizing DNA & RNA and offers superior sensitivity and performance to current market leaders. It is a food-grade safe Minor Groove Binder that does not distort DNA structures & inhibit PCR as seen with intercalating DNA stains such as Ethidium Bromide and SYBR group of dyes. **tinto rang™** is compatible with all existing gel documentation systems and can even be used for isothermal applications. Optimal performance is achieved by pre-loading **tinto rang™** relative to pre-casting or post-staining.



WHY **tinto rang™**?

Superior performance and ease of use

- Compatibility with all existing gel documentation, (RT)qPCR, and LAMP systems
- 100x more sensitive than SYBR Safe
- Stable for up to 150 days at room temperature
- No PCR inhibition

Relegate bio-hazard disposal costs to the past

 Certified food grade safe dye

 Safe disposal - Eco-flours can be directly disposed into waster water systems

 No PPE required

 Spillage carries zero risk

FEATURES



One minute DNA Stain



Non - Mutagenic food
graded stain



Sensitivity of 25 picogram



Minor Groove Binder



SPECTRAL PROPERTIES

Molecular Coefficient

16000

Quantum Yield

0.965

Excitation wavelength

290, 490 & 530nm

Emission wavelength

570nm

 **10000 FOLD INCREASE IN FLUORESCENCE**

10000 fold enhancement of fluorescent intensity on binding to CT DNA

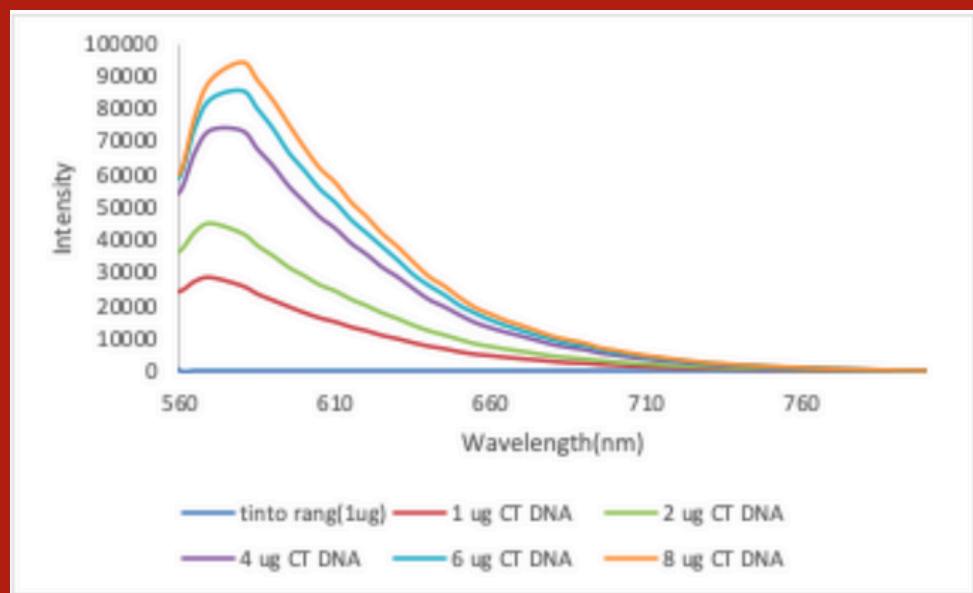


Fig.1: Concentration dependent enhancement in fluorescence of the dye candidate upon binding to CT DNA

VS COMPARISON OF **tinto rang™** WITH MARKET LEADERS

THE PICOGRAM COMPARISON

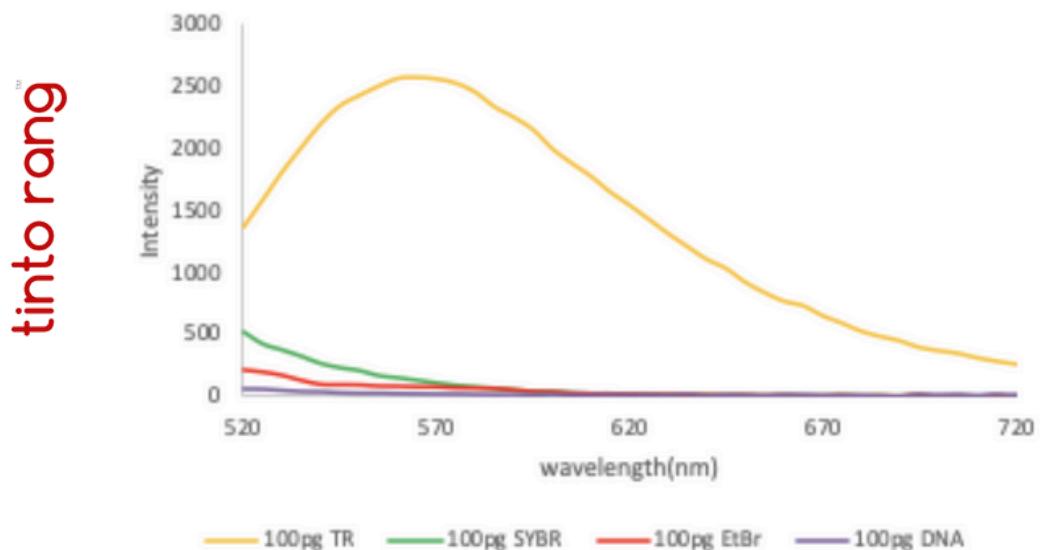


Fig. 2: Comparison of sensitivity (picogram) of **tinto rang** candidates with **SYBR Green** and **Ethidium bromide**


COMPARISON WITH MARKET LEADERS

NUCLEIC ACID STAINS	tinto rang™	ETBR	SYBR GREEN
 Safety	Food Grade safe	Carcinogenic, less Mutagenic	Carcinogenic, Mutagenic, Teratogenic
 Speed/ Time	Binds to DNA in 1 minute	20-40 minutes	20-40 minutes
 Sensitivity	100pg	3ng	1-5ng
 Binding Mode	Minor Groove Binder	Intercalator	Intercalator
 Quantum yield	0.95	0.7	0.2
 PCR Inhibition	No	Yes	Yes



TINTO RANG'S NON-INHIBITORY POWER

PCR based application (tinto rang™ does not inhibit PCR)

Dye based readymade PCR master-mixes are potent product in labs where molecular research is carried out. Most of the fluorescent dyes inhibit PCR at higher concentrations. On testing, we found that tinto rang (STR) does not inhibit PCR even at high concentrations unlike the other two dyes - EtBr and SYBR. The dye is now being explored for applications in Q-PCR based assays.



Fig.3 : PCR amplified products (800bp). No amplification seen in the presence of EtBr and SYBR Green in the mastermix STR- tinto rang



TINTO RANG'S NON-TOXIC BRILLIANCE

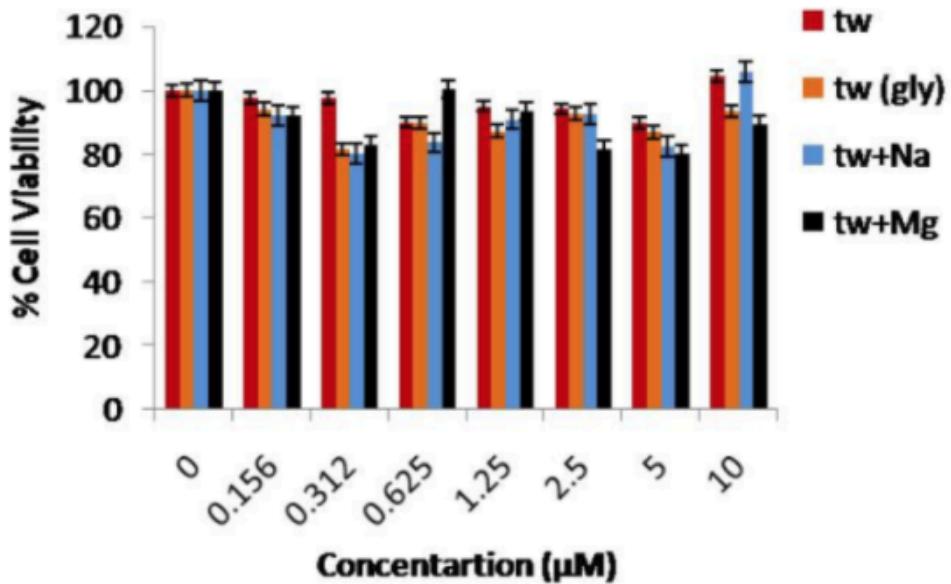


Fig.4: MTT assay with different concentrations of tinto rang

MTT assay showed more than 95 % growth in the cells even at concentrations as high as 10 μM and established the non toxic property of tinto rang.



THE MINOR GROOVE BINDER

Mode of Binding Studies

Circular Dichroism

The CD spectra reveals that the binding of the dye does not distort the secondary structure of the DNA molecule.

CD spectra of DNA before and after dye binding

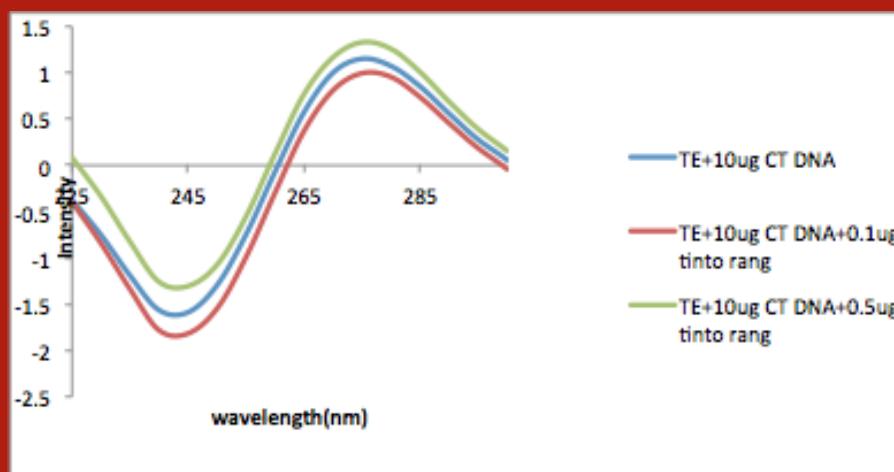


Fig 5: Dye displacement studies with Ethidium Bromide and SYBR green and have illustrated the fact that tinto rang is not an intercalator and does not compete with EtBr or SYBR during binding. The results indicate that tinto rang is more of a groove and or external binder (under publication process).

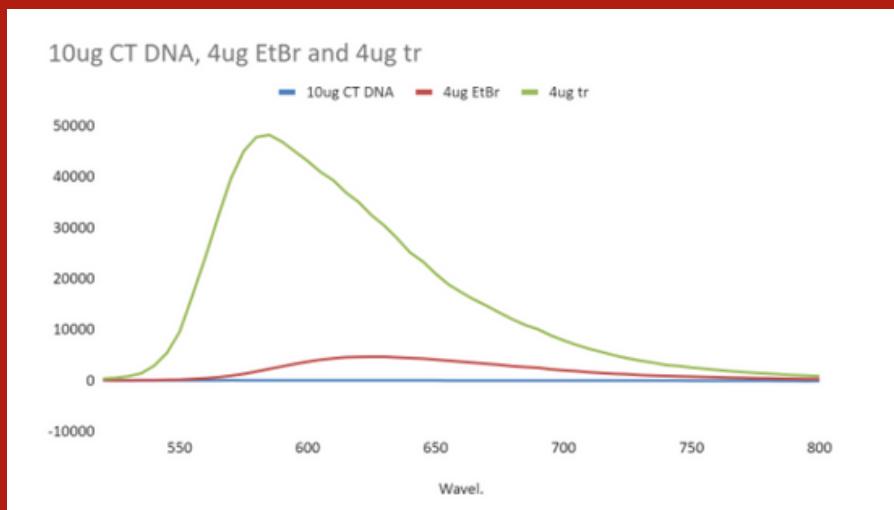


Fig.6: Dye displacement with EtBr shows that binding of tinto rang is independent of EtBr and hence not intercalation. Further it also shows a very good fluorescence enhancement with TR when compared to EtBr.



DOCKING AND MOLECULAR SIMULATION STUDIES

Molecular simulations demonstrated that tinto rang is a minor groove binder predominantly and it does not intercalate even if placed forcefully. Our studies show that tinto rang binds to the minor groove more strongly than the major groove.

Docking Study

- Two different algorithms were used for the docking (i) Genetic Algorithm (GA), (ii) Simulated Annealing (SA).
- The results show that the groove binding is preferred over intercalation.

Algorithm	BE (kCal/mol) Minor	BE (kCal/mol) Major
GA	-6.10	-4.62
SA	-5.10	-3.30





TINTO RANG IN ISOTHERMAL AMPLIFICATION (LAMP)

SYBR green and ethidium bromide have been reported to be strong inhibitors of BST Polymerase, tinto rang does not inhibit BST Polymerase.

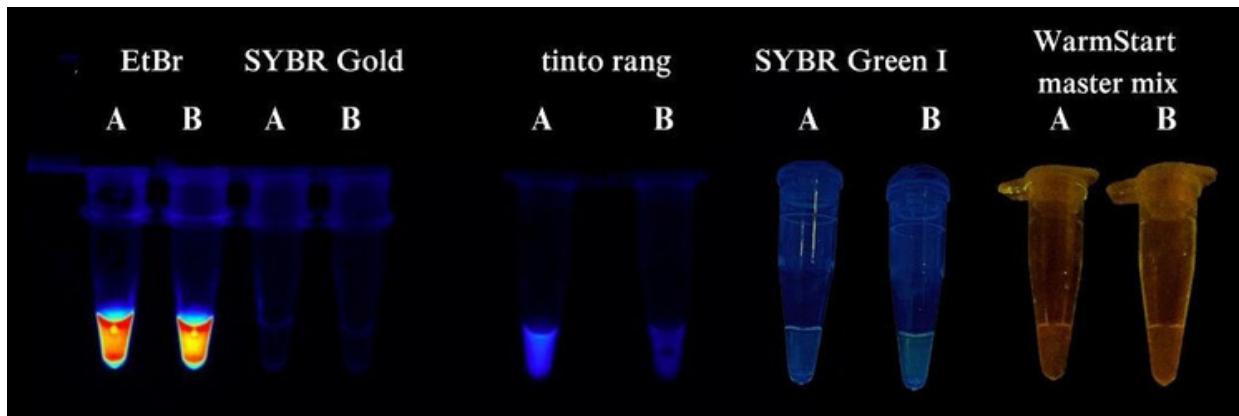


Fig 7: A-Positive template, B-NTC-Comparison of change in fluorescence between positive template & NTC after LAMP reaction with different dyes

1. EtBr- Similar fluorescence in both positive template & NTC,
2. SYBR gold- no fluorescence.
3. tinto rang- visible change in fluorescence between positive template & NTC.
4. SYBR Green I- Brighter fluorescence in NTC compared to positive template.
5. WarmStart 2x master mix- no fluorescence.



INSTRUCTIONS

tinto rang™ For LAMP

- **Concentration** 10X/ μ L
- **Working Conc**
 - 10X / reaction
 - Take 1 μ L from 10X stock per reaction

NOTE: Use LAMP diluent provided with the kit for any dilution purposes

tinto rang™ For RT PCR

- **Concentration** 20,000X & 10,000X
- **Substock Preparation** For 100 reactions- 10 μ L from stock + 90 μ L of RT PCR Diluent
- **Working Conc** 1 μ L from substock per reaction

NOTE: Use RT-PCR diluent provided with the kit for any dilution purposes



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