DuraScribe T7 Transcription Kit

Lucigen

DuraScribe®

10 Reaktionen

Artikel-Nr.: 150310 | Lucigen | Hersteller-Nr.: DS010910

598,40 €*

*zzgl. MwSt. zzgl. Versandkosten

Beschreibung

Besonderheit: Nuclease Resitente RNA
Produkttyp: IVT Kit
Verpackung: 10 Reaktionen

Mit dem DuraScribe T7 Transcription Kit wird in hoher Ausbeute sehr stabile RNA synthetisiert. Die sog. DuraScribe RNA ist vollständig resistent gegenüber RNase A und verwandten, häufig in Laboratorien vertretenen Ribonukleasen. DuraScribe RNA behält jedoch die Sensitivität gegenüber anderen RNasen, wie z.B. RNase T1 und RNase H. Ermöglicht wird dies durch den Einbau von 2´-F-CTP und 2´-F-UTP in der DuraScribe *in vitro* Transkriptionsreaktion. Die DuraScribe T7 RNA Polymerase verwendet den gleichen T7 Promotor wie die Standard T7 RNA Polymerase.

Die Vorteile:

- Einbau von 2’-F-CTP und 2’-F-UTP während der *in vitro* Transkriptionsreaktion
- DuraScribe RNA ist vollständig resistent gegenüber RNase A und verwandten Ribonukleasen
- Weiterhin sensitiv gegen z.B. RNase T1, RNase H
- Verwendung des Standard T7 Promotors durch die DuraScribe T7 RNA Polymerase

Typische Anwendungen:

- *In situ* Hybridisierung
- Ribonuclease Protection Assays
- RNA Interference (RNAi)
- Antisense RNA

Inhalt des Kits:

- Durascribe T7 Enzym Mix
- Durascribe 10 x Reaktionspuffer

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- ATP
- GTP
- 2'-F-CTP
- 2'-F-UTP
- DNase I
- DTT (Dithiothreitol)
- Kontrolltemplate DNA (linearisiert)
- Wasser (RNase-frei)

Table 1. Yield of DuraScript® RNA from a DuraScribe® Kit reaction. One microgram of a 3-Kb DNA template was linearized at different sites and then transcribed in a DuraScribe T7 Transcription Kit reaction for 4 hours. The yield of DuraScript RNA produced from each template is shown in micrograms (µg) and in picomoles (pmol).

Size of DuraScript RNA produced	DuraScript RNA Yield (µg)	DuraScript RNA Yield (pmol)
2600 nts	100 µg	116 pmol
1400 nts	58 µg	124 pmol
330 nts	18 µg	164 pmol
88 nts	9 µg	307 pmol

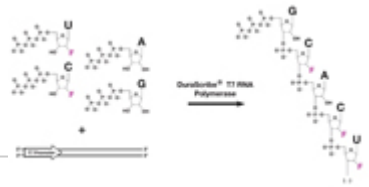


Figure 1 (click to enlarge). The DuraScribe® T7 RNA Polymerase efficiently incorporates 2´-F-dCTP and 2´-F-dUTP into full-length DuraScript® RNA. The presence of the fluorine at the 2´-position of the 2´-F-dC and 2´-F-dU nucleotides prevents digestion by RNase A.

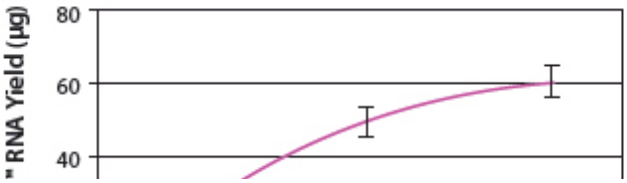


Figure 2. Yield of RNA from a DuraScribe® T7 Transcription reaction. A standard reaction (4-6 hours) produced 40-60 µg of a 1.4-kb DuraScript® RNA.

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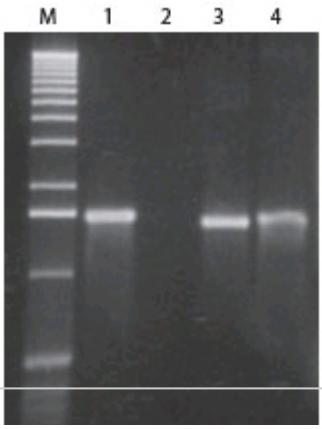


Figure 3. DuraScript® RNA is resistant to RNase A digestion. A 1.4-kb standard RNA transcript and a 1.4-kb DuraScript RNA transcript were each incubated with 1 U of highly purified RNase A for 30 minutes. The standard RNA transcript was completely degraded while the DuraScript RNA transcript remained intact. Lane M, size ladder; lane 1, 1.4-kb standard RNA transcript; lane 2, standard RNA after RNase A treatment; lane 3, 1.4-kb DuraScript RNA; lane 4, DuraScript RNA after RNase A treatment.

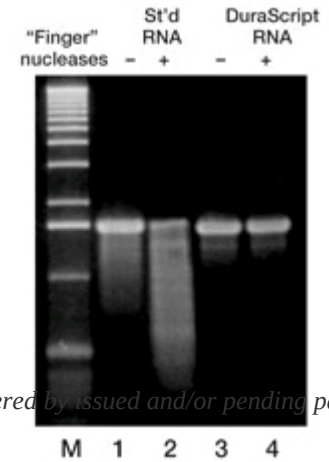


Figure 4. DuraScript® RNA is completely resistant to "finger" nucleases. A 1.4-kb standard RNA transcript and a 1.4-kb DuraScript RNA transcript were produced using sterile water or water that had been contaminated by exposure to the hands of a test subject. The standard RNA shows extensive degradation from "finger" nucleases in the contaminated water while the DuraScript RNA remains fully intact. M, Size ladder; Lane 1, Standard RNA transcript; Lane 2, Standard RNA after "finger" nuclease exposure; Lane 3, DuraScript RNA; Lane 4, DuraScript RNA after "finger" nuclease exposure.

** Covered by issued and/or pending patents.*

References

1. Sousa, R. and Padilla, R. (1995) EMBOJ. 14:18 4609-4621.

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