Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific PowerCut Dicer

#F-602S 60 U

Lot _ Expiry Date _

Ordering information

Component	#F-602S	#F-602L
PowerCut Dicer, 1 U/µL	60 U (60 µL)	300 U (300 µL)
5X PowerCut Dicer Reaction Buffer	500 µL	500 µL

Store at -20°C

www.thermoscientific.com/onebio

Description

Thermo Scientific PowerCut Dicer is a recombinant endoribonuclease originating from *Giardia intestinalis.* It cleaves dsRNA to small interfering RNA (siRNA) with a 100% efficiency. The produced siRNA fragments have a length of 25–27 nucleotides, and are capable of triggering RNA interference when transfected into cells.

Definition of Activity Unit

One unit is defined as the amount of enzyme that is needed to completely cleave 1 µg of 192 bp double-stranded RNA substrate to siRNA in 16 hours at 37°C.

Source

PowerCut Dicer enzyme is purified from an *E.coli* strain expressing the cloned PowerCut Dicer gene from *Giardia intestinalis.*

Storage buffer: 50 mM Tris-HCl pH 8 (25°C), 50 mM NaCl, 5 mM $MgCl_2$, 0.2 mg/ml BSA, 50% glycerol.

Reaction buffer: 5X PowerCut Dicer Reaction Buffer.

Rev.2

Guidelines for using PowerCut™ Dicer Reaction protocol

Make sure to avoid contaminating RNases when setting up the reactions.

Table 1. Pipetting instructions.

Component	20 µL rxn	Final concentration
H_2O (RNase free)	Add to 20 µL	
5X PowerCut Dicer Reaction Buffer	4 µL	1X
dsRNA	X µL	4 µg
PowerCut Dicer (1 U/µL)	4 µL	4 U

Incubate at 37°C for 16 h, preferably in a heat block.

Purify the siRNA using standard methods.

Agarose gel analysis of the reactions

Figure 1. A dicing reaction was performed using dsRNA from 193 bp furin, 812 bp eGFP and phi6 as substrates. The reactions were assembled and incubated according to the standard protocol, and unpurified aliquots were run on a 2% agarose gel. No uncut substrate is seen in the reactions demonstrating 100% cleaving efficiency. M denotes a 27 bp RNA marker.

Exonuclease contamination assay: Incubation of 1 U of PowerCut Dicer (4 h, 37°C, 50 µL) with 1 µg of sonicated [^{3}H]-DNA (2×10^5 cpm/µg) in the assay buffer released <0.5% of radioactivity.

Endonuclease contamination assay: Incubation of 1 U of PowerCut Dicer (4 h, 37°C, 50 µL) with 1 µg of ΦX174 RFI DNA in the assay buffer resulted in <10% conversion to RFII form.

Ribonuclease contamination assay: Incubation of short ssRNA in the presence or absence of 1 U of PowerCut Dicer (1 h, 37°C, 20 µL) in the assay buffer produced similar results.

Technical support
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