

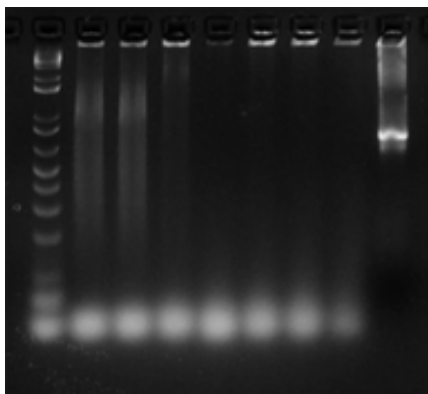
Recombinant Human Dicer Enzyme Kit

PRODUCT SUMMARY

Cat. No: T510002 (50 Units) / T510008 (4 x 50 Units)

Description: The Recombinant Human Dicer Enzyme Kit uses an ultra-active form of human recombinant dicer enzyme that can cleave more than 95% of dsRNA template into 22 bp siRNAs within 12 hours under optimized reaction conditions (Figure 1). This provides sufficient siRNAs in every single species to achieve effective gene silencing.

Fig. 1 Efficient Digestion of Double-Stranded RNA with Recombinant Human Dicer Enzyme



1 µg of a 700 bp dsRNA after 12 hour of digestion by the dicer enzyme. Lanes: 1. marker; 2. 1 µl dicer, 3. 1.5 µl dicer, 4. 2 µl dicer, 5. 2.5 µl dicer, 6. 3 µl dicer, 7. 3.5 µl dicer, 8. 4 µl dicer, 9. 1 µg dsRNA (control)

Please note: The Recombinant Human Dicer Enzyme Kit is intended FOR RESEARCH USE ONLY.

Components:

Description	Quantity
Recombinant Dicer Enzyme (0.5 unit/µl)	1 tube x 100 µl (Cat. #T510002) 4 tubes x 100 µl (Cat. #T510008)
Dicer Reaction Buffer	1 tube x 200 µl (Cat. T510002) 4 tubes x 200 µl (Cat. #T510008)
50 mM MgCl ₂ Solution	1 tube x 25 µl (Cat. #T510002) 4 tubes x 25 µl (Cat. #T510008)
10 mM ATP	1 tube x 50 µl (Cat. #T510002) 4 tubes x 50 µl (Cat. #T510008)
Dicer Stop Solution	1 tube x 100 µl (Cat. T510002) 4 tubes x 100 µl (Cat T510008)
Nuclease-free Water	1 tube x 1 ml (Cat. #T510002) 4 tubes x 1 ml (Cat. #T510008)

Shipping and Storage: The Recombinant Human Dicer Enzyme Kit is shipped frozen. For maximum stability and long-term use, immediately store at -20°C upon receipt. All components are stable for six months when stored properly.

METHODS AND PROCEDURES

Preliminary Notes

1. We recommend that you use dsRNA templates of 500 – 1000 bp in length. The dicer enzyme might not digest well if the dsRNA is smaller than 300 bp.

2. Double-stranded RNA generated using the TurboScript™ T7 RNA Transcription Kit can be used directly for the Recombinant Dicer Enzyme Kit without purification. However, dsRNA purified using standard lithium chloride purification procedures can give slightly better results.

3. For first time users, we strongly recommend using the Dicer siRNA Generation Kit (Cat. # T510001)

Generation of siRNA Using Recombinant Dicer Enzyme

1. *Keep the Dicer Reaction Buffer at room temperature while assembling the reaction.* The following amounts are for a single 10 µl reaction.

2.5 µl –x	Nuclease-free water
x µl	dsRNA (1 µg)
1 µl	10 mM ATP
0.5 µl	50 mM MgCl ₂
4 µl	Dicer Reaction Buffer
2 µl	Recombinant Dicer Enzyme (1 Unit)

NOTE 1 µg of dsRNA control template will yield about 0.5 µg of d-siRNA, which is sufficient for one or two transfections in 24-well plates using the GeneSilencer siRNA Transfection Reagent. Adjust the reaction volume accordingly if you need more d-siRNAs.

IMPORTANT Avoid using excess recombinant dicer enzyme, as it may decrease the amount of diced-siRNA (dsiRNA) that can be generated from the digestion reaction.

- Incubate overnight (12 to 18 hours) at 37°C.
- Stop the reaction by adding 2 µl Dicer Stop Solution.
- Check d-siRNA (~22 base pairs) by using one of the following methods:
 - 3 % agarose Gel (TAE)
 - 15% native polyacrylamide gel (29:1, cast in 1X and electrophoresed in 0.5X TBE). Run at 10 Watts and 4°C. Visualize RNA by staining with ethidium bromide.

TROUBLESHOOTING GUIDE

Problem	Possible Causes	Recommended Solutions
d-siRNA yield is low.	No dsRNA or poor dsRNA quality.	Check the amount of dsRNA added. Make sure that the correct fractions from the RNA Purification Columns are used and/or purify dsRNA before dicer reaction.
	Too much Recombinant Dicer Enzyme was added.	Use 1 unit of the enzyme for every microgram of dsRNA.
	10 mM ATP is old.	Use fresh ATP solution.

RELATED PRODUCTS

Product	Cat. No.
<i>For efficient generation of siRNA</i> Dicer™ siRNA Generation Kit 5 genes / 50 transfections	T510001
TurboScript™ T7 Transcription Kit 20 Reactions	T510003
RNA Purification Column 1 20 Columns	T510004
RNA Purification Column 2 20 Columns	T510005
<i>For efficient and functional siRNA transfection</i> GeneSilencer™ siRNA Transfection Reagent 200 reactions (0.75 ml)	T500750
GeneSilencer™ siRNA Transfection Reagent 5 x 200 reactions (5 x 0.75 ml)	T505750
<i>For efficient transfection of DNA into diverse cell lines</i> GenePORTER™ 2 Transfection Reagent 75 reactions (0.75 ml)	T202007
GenePORTER™ 2 Transfection Reagent 150 reactions (1.5 ml)	T202015
GenePORTER™ 2 Transfection Reagent 750 reactions (5 x 1.5 ml)	T202075
<i>For 3-minute transformation into E. coli</i> TurboCells™ Chemically Competent <i>E. coli</i> 20 x 50 µl	C300020
TurboCells™ F' Chemically Competent <i>E. coli</i> 20 x 50 µl	C301020

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Limited Label License for the Recombinant Dicer Enzyme

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Please contact us for a complete list of gene therapy research products.

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