

Test plate for *Drosophila* dsRNA Library

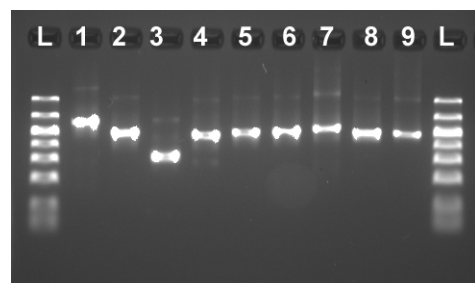
Catalog Number: RDM4429

Product Description

The test plate containing dsRNAs for the *Drosophila* dsRNA library is represented by a total of 8 genes with well-known and easily identifiable RNAi phenotypes as well as the non-silencing control. Each dsRNA is provided in nuclease-free buffer (10mM Tris pH 7.0, 1mM EDTA) at concentration 0.45µg/µl. Each well contains 12µl +/-1ul of the RNA solution.

Genes represented in the test plate

Gene name	CG#	Location in the library	RNAi phenotype
DIAP1	12284	62- A12	Cell death
RpL1	5502	23- F7	Low cell number
RpS18	8900	44- C9	Low cell number
SCAR	4636	18- D9	Loss of actin from cellular cortex
Polo	12306	62- B11	Accumulation of metaphases
Pavarotti	1258	1- H11	Bi-nuclear cells
Pebble	8114	39- E7	Bi-nuclear cells
RacGAP50C	13345	64- D12	Bi-nuclear cells

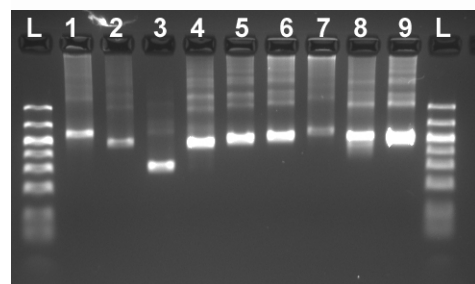


L- 1 kb ladder, 1- DIAP1, 2- RpL1, 3- RpS18, 4- SCAR, 5- pav, 6- pebble, 7- rac1GAP50C, 8- polo, 9- non silencing control

DNA

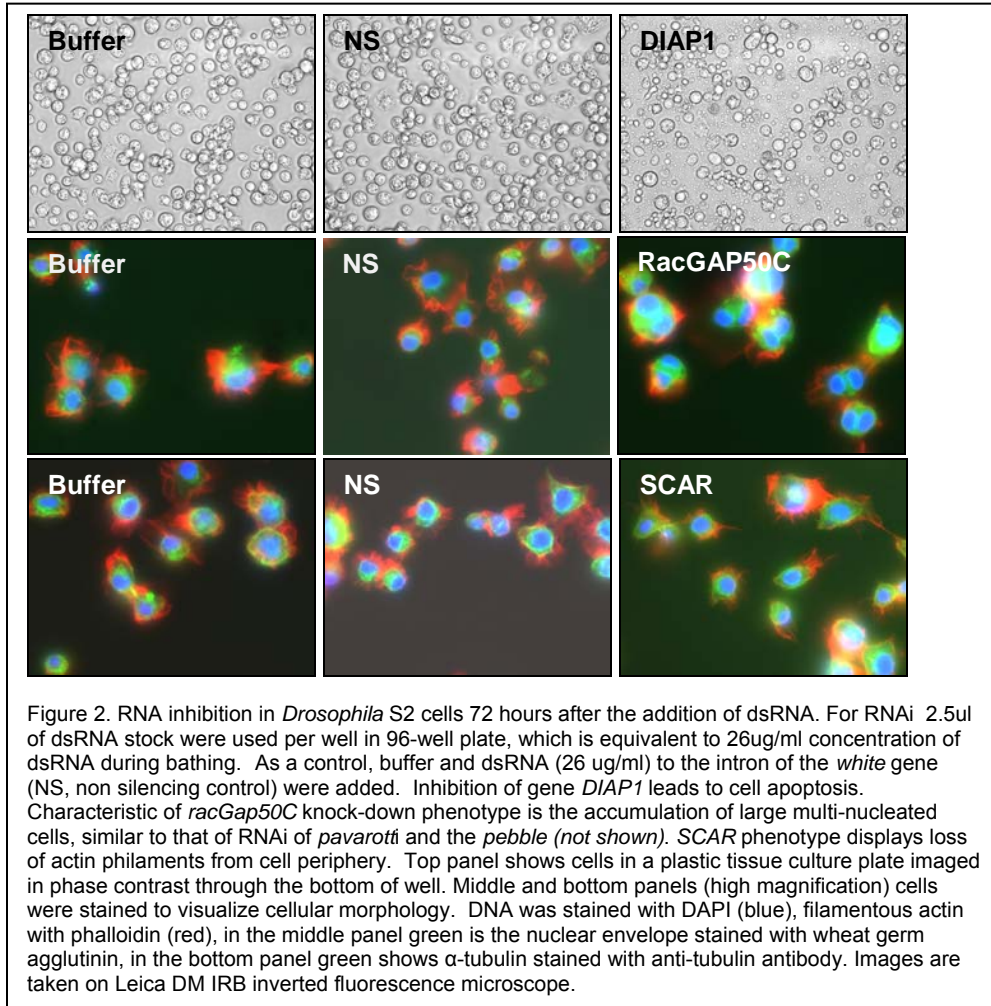
Figure 1. Gel images of DNA and dsRNA constructs from the *Drosophila* RNAi test plate

Molecular weight ladder (L), *DIAP1*(1), *RpL1* (2), *RpS18* (3), *SCAR* (4), *pav* (5), *pebble* (6), *racGAP50C* (7), *polo* (8) and NS control (9) dsDNA and dsRNA separated on 2% non-denaturing TBE agarose gel. Note the majority of RNA is present in a double-stranded form. When more than two strands of RNA anneal together, they form ladder-like smear in a higher molecular weight area. Presence of these forms of RNA in the dsRNA preparations does not adversely affect their silencing ability. Approximately 150ng of RNA was loaded into each well. RNA is visualized by SybrGreen1 staining.



dsRNA

RNAi phenotypes using dsRNA constructs from the test plate



Recommended RNAi procedures

Methodological Papers:

Carolyn A. Worby, Nancy Simonson-Leff, and Jack E. Dixon (2001) RNA Interference of Gene Expression (RNAi) in Cultured *Drosophila* Cells. *Sci. STKE* (95), pl1. [DOI: 10.1126/stke.2001.95.pl1]

Armknecht S, Boutros M, Kiger A, Nybakken K, Mathey-Prevot B, Perrimon N. (2005) High-throughput RNA interference screens in *Drosophila* tissue culture cells. *Methods Enzymol.*; 392: 55-73

Christophe J. Echeverri and Norbert Perrimon.(2006) High-throughput RNAi screening in cultured cells: a user's guide. Nature Reviews Genetics 7, 373-384.

Please see guidelines below for setting up your RNAi experiments using the dsRNA library:

COMPONENT	96-WELL	384-WELL
dsRNA	2-3 ul	0.5-0.75 ul
Media with cells	40 ul	10 ul
Media/10%FBS	80 ul	20 ul

For aliquoting convenience the RNA stock can be diluted. To dilute the RNA, add equal amount of DEPC-treated water to all the wells in a plate, mix thoroughly and aliquot for your experiment.

RNA can be added to a sterile tissue culture plate first, followed by addition of cells resuspended in the media without serum.

Incubate cells with dsRNA for 30-40 minutes prior to addition of media with serum. It is generally recommended to examine RNAi phenotype after 72hours.

Useful websites

Flybase: A database of the *Drosophila* Genome <http://flybase.bio.indiana.edu>

The Berkeley *Drosophila* Genome Project <http://www.fruitfly.org>

FLIGHT database of data from high-throughput experiments carried out in *Drosophila* cell culture. <http://www.flight.licr.org/>

Drosophila RNAi Screening Center <http://www.flyrnai.org>

Drosophila RNAi References

Cheng LW, Viala JP, Stuurman N, Wiedemann U, Vale RD, Portnoy DA. (2005) Use of RNA interference in *Drosophila* S2 cells to identify host pathways controlling compartmentalization of an intracellular pathogen. Proc Natl Acad Sci USA.;102(38):13646-51

Meister G, Tuschl T.(2004) Mechanisms of gene silencing by double-stranded RNA. Nature, 431(7006):343-9.

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Schmid A, Schindelholz B, Zinn K. (2002) Combinatorial RNAi: a method for evaluating the functions of gene families in *Drosophila*. *Trends Neurosci* 25(2):71-74.

Celotto A.M., Graveley B.R. (2002) Exon-Specific RNAi: A Tool for Dissecting the Functional Relevance of Alternative Splicing, *RNA* 2002, 8(6): 718-724.

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Kennerdell JR, and Carthew RW. (2000) Heritable gene silencing in *Drosophila* using double-stranded RNA. *Nature Biotech* 18: 896-898.

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