Feb 14, 2019 Version 3

Transfection of constructs in diplonemids to enhance the HR pathway, using RS-1 (3-(benzylamino) sulfonyl)-4-bromo-N-(4-bromophenyl) benzamide). V.3

DOI
dx.doi.org/10.17504/protocols.io.x45fqy6

Binnypreet Kaur[1], Drahomíra Faktorová[2], Julius Lukeš[3]

[1][Institute of Parasitology Czech Academy of Sciences Faculty of Sciences University of South Bohemia Czech Republic];
[2][Institute of Parasitology Czech Academy of Sciences Faculty of Sciences University of South Bohemia Czech Republic];
[3][Institute of Parasitology Czech Academy of Sciences Faculty of Sciences University of South Bohemia Czech Republic] [Canadian Institute for Advanced Research Toronto Canada]

Protist Research to Opti... Julius Lukes

Binnypreet Kaur
1 Institute of Parasitology, Biology Centre, Czech Academy o...

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.x45fqy6

Protocol Citation: Binnypreet Kaur, Drahomíra Faktorová , Julius Lukeš 2019. Transfection of constructs in diplonemids to enhance the HR pathway, using RS-1 (3-(benzylamino) sulfonyl)-4-bromo-N-(4-bromophenyl) benzamide).. **protocols.io https://dx.doi.org/10.17504/protocols.io.x45fqy6**

Protocol status: In development
We are still developing and optimizing this protocol

Created: February 14, 2019

Last Modified: February 14, 2019

Protocol Integer ID: 20349

Abstract

RS-1 (RAD51-stimulatory compound 1) is a stimulator of homologous recombination (HR) protein RAD51

1 Step 1: Countthe cells and plan to 1 nucleofection with 5× 10^7 cells for each construct.

2 Step 2: Harvestthe cells by centrifugation at 1300xg for 5 min at room temperature in Swing Bucket Rotor.

3 Step 3: Resuspendthe cell pellet in 100ul of AMAXA Human T- cell solution at 4C (from refrigerator combine 81.8ul of Human T-cell nucleofectorsolution + 18.2ul Supplement).

4 Step 4: Add 5-10ug of (PCR) (linearized DNA) along with 0.3 uM RS-1 enhancer into the cuvette (resuspend in 10ul of H2O).

5 Step 5: Put everything into the cuvette, close the cap and place in the electroporator, cuvette should only fit in one direction, but metal sides should face towards you.

6 Step6: Press for the Program X-001 to electroporate and repeat the same as mentioned in tranformation protocol.

7