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

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Binnypreet Kaur¹, Drahomíra Faktorová², Julius Lukeš³

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...





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

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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.

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