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DATA SHEET

Reagent:	HIV-2 GST-Tat Expression Vector (46-130)
Catalog Number:	3643
Lot Number:	7/15/97
Release Category:	A
Provided:	Each clone provided as 1 ml ampicillin-resistant, transformed bacteria.
Cloning Site:	BamHI–EcoRI.
Cloning Vector:	pGEX2T.
Description:	These constructs contain mutated HIV-2 tat genes. A thrombin proteolytic cleavage site is located between the Schistosoma japonicum glutathione S-transferase (GST) sequence and the tat insert.
Special Characteristics:	Mutated HIV-2 Tat is expressed as a GST-Tat fusion protein, which can be easily expressed as described in the attached protocol. Alternate names include: GST-Tat2 (46-130)
Recommended Storage:	-70°C.
Contributor:	Dr. Sandra Tong-Starksen.
References:	Pagtakhan AS, Tong-Starksen SE. Interactions between Tat of HIV-2 and transcription factor Sp1. *Virology* **238**:221-230, 1997.

NOTE:

Acknowledgment for publications should read "The following reagent was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: HIV-2 GST-Tat Expression Vector (46-130) from Dr. Sandra Tong (cat# 3643)." Also include the reference cited above in any publications.

Limited to one aliquot per laboratory.

Research Chart:

Clone	Cat. No.	Description
GST-Tat2 (R81–84A)	3642	Two exons; substitution mutation of the basic residues (R81-84) to alanine in full-length tat2 cDNA.
GST-Tat2 (46–130)	3643	Two exons; aa 1-45 deleted.
GST-Tat2 (1–80)	3644	One exon; aa 81-130 deleted.

Preparation and Purification of HIV-2 Tat from Bacterial Expression Systems

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Reagents:

LB-amp Medium	Luria Broth containing 50 µg/ml ampicillin
GST-Tat Expression Plasmids	Catalog #3642–3644
IPTG	Isopropylthio-β-galactoside (BRL Catalog #5529). Make 100 mM stock solution using distilled water.
EBC Buffer	50 mM Tris-HCl (pH 8.0), 120 mM NaCl, 0.5% NP-40
EBC-DTT Buffer	EBC containing 5 mM DTT (make fresh)
EBC-DTT-SDS Buffer	EBC-DTT buffer containing 0.075% SDS (make fresh)
Protease Inhibitors	Prepare at the following final concentrations: Aprotinin (2 µg/ml), Leupeptin (1 βg/ml), PMSF (50 βg/ml). Stocks should be made fresh in 4 ml final volume (see step 6 below).
Lysozyme	16 mg/ml stock
Glutathione Sepharose beads	Pharmacia Catalog #17-0756-01
Thrombin Cleavage Buffer	50 mM Tris-HCl (pH 7.6), 20 mM KCl, 1 mM DTT
Human Thrombin	Sigma catalog #T-0310 (supplied as a resuspension)
3X Freeze Solution	60% glycerol containing 15 mM DTT (make fresh)
Other Reagents	SDS-PAGE Gel and buffers; Coomassie blue; protein standard markers

Culture and Preparation of GST-Tat Extracts:

1. Inoculate 50 ml of LB-amp medium with the glycerol stock of E. coli harboring the desired GST-Tat expression plasmid.

2. Place the culture in a shaking 37°C incubator and leave overnight.

3. The next day, dilute the culture 1:10 in LB-amp to give 500 ml final volume. Continue the incubation for 3 more hours.

4. Add 0.5 ml of IPTG to give a 0.1 mM final concentration. Continue the incubation for 1.5 more hours.

5. Transfer the culture to centrifuge tubes and centrifuge at 5000 rpm for 10 minutes at 4°C.

6. Pour the medium off and retain the bacterial pellet. Resuspend the pellet in 4 ml of EBC-DTT buffer containing protease inhibitors, and 2 mg/ml lysozyme, if desired. Note that lysozyme should not be used if the GST-Tat preparation is to be used in RNA binding experiments, as there is some concern that residual lysozyme may interfere with RNA binding.

7. Place the tubes on ice for 15 minutes.

8. Sonicate the tubes for 30 seconds, and repeat twice. Place the tubes on ice between the sonications.

9. Transfer the solubilized bacteria to 1.5 ml microfuge tubes. Centrifuge at 12,000 rpm for 15 minutes at 4°C using a microfuge.

10. Transfer the supernatant to fresh tubes and make 1 ml aliquots. The supernatant can be stored at -70°C, and will remain stable for at least one year at this temperature.

Estimation of Protein Concentration:

1. Thaw the stock supernatant by placing the tube briefly at 37°C.

2. Add 250 µl EBC-DTT Buffer to 50 µl of each *E. coli* extract.

3. Add 25 µl of equilibrated glutathione-sepharose beads. [Equilibrate beads as follows: Pellet the beads (supplied by the manufacturer in 80% solution) by centrifuging at 12,000 rpm for 10 seconds in a microfuge. Pour off the supernatant. Add 500-1000 µl EBC-DTT, invert the tube several times (do not vortex). Pellet the beads as described above and repeat the washing. Resuspend the final bead pellet to give a 50% suspension in EBC-DTT.]

4. Incubate the samples 15-30 minutes on a rocker placed in a cold room (4°C).

5. Wash the beads by centrifuging for 10 seconds at 12,000 rpm in a microfuge, discard the supernatant, then add 500 µl of EBC-DTT-SDS buffer and invert the tube several times. Repeat the centrifugation and wash step one time.

6. Resuspend the final pellet in volume of SDS-PAGE buffer.

7. Load the samples onto a 15%DSD PAGE gel with 2.5 µg marker standards. If desired, load 1 µl of the unbound E. coli extracts.

8. After completing the electrophoresis, stain the gel with Coomassie blue.

9. Estimate the protein concentration by comparing the GST-Tat bands with marker standards and/or previous preparations.

Thrombin Digestion:

1. Transfer the beads into a 15 ml conical tube and add 50-fold volume of thrombin cleavage buffer. Centrifuge the sample at 3000 rpm for 3 minutes at room temperature.

2. Discard the supernatant and resuspend the pellet in 100 µl of thrombin cleavage buffer per ml of *E. coli* lysate + 10 µl (equal to 5 units or 1.5 µg) of the human thrombin. Incubate at room temperature for 1 hour with gentle mixing.

3. To elute the cleaved Tat protein from the GST-bound sepharose, incubate the beads at 37°C for 3 minutes then briefly centrifuge the sample at 12,000 rpm for 30 seconds at room temperature using a microfuge.

4. Collect the eluate and repeat step 3 two times.

5. Pool the protein eluates. Adjust their concentrations in 1X freeze solution to give final concentrations of 20% glycerol and 7 mM DTT. Store the preparations at -70°C.

Last Updated: November 27, 2018