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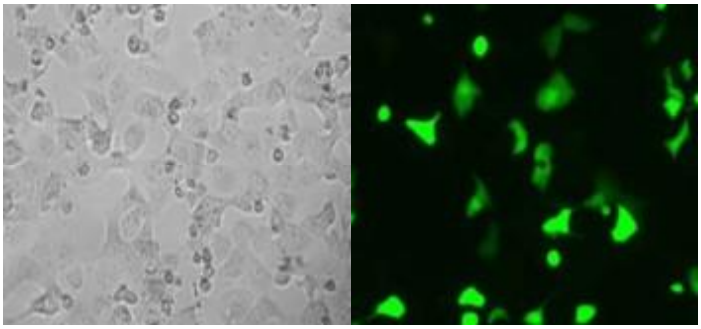
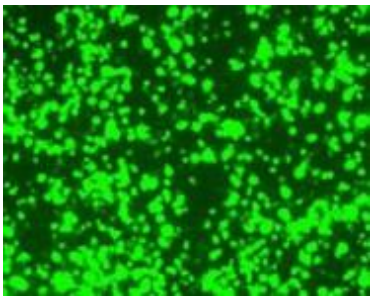
Targefect-BAC



Upper Panel:
Transfection of 170 Kb BAC expression vector expressing green fluorescent protein into Vero cells using the Targefect-BAC kit (combination of Targefect-BAC and virofect).



Lower Panel:
Transfection of HEK-293 cells with BAC DNA using the Targefect BAC reagent (without Virofect) Data courtesy of Dr. Fuchen Zhou and Dr. S. Gao, University of Texas Health Science Center at San Antonio, TX

The Targefect-BAC transfection kit has 3 components

Targefect-BAC- Transfection reagent

Vriofect Enhancer- An adenovirus derived enhancer formulations that enhances BAC delivery by exploiting adenoviral receptors on the cell surface

Peptide Enhancer: The peptide enhancer is a modified protein based formulation which enhances gene transfer by increasing transgene delivery to the nucleus when used in combination with the Targefect reagents. Once the transfection complex is internalized, the peptide enhancer helps the transfection complex escape degradation in the lysosome and enhances the duration of transgene expression.

Two protocols are provided for transfection of BAC DNA

One protocol uses the Targefect-BAC reagent in combination with the Virofect enhancer and a second protocol uses the Targefect-BAC reagent with the Peptide enhancer. We recommend the end user try both protocols as there is a lab-to-lab variation as to which protocol is preferred

Set up cells to be transfected so that they are about 70% confluent at the time of the experiment.
Store Virofect at -20oC or -70 o C. Store the Targefect-BAC reagent at 4oC. Do not vortex it and freeze it.
Prepare transfection complexes as follows: Use clear plastic tubes for complex formation.

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Complexing conditions: USE HIGH GLUCOSE DMEM (Dulbecco's modified eagle's medium containing 4500 mg/liter glucose). Make additions as follows -
Add DMEM first, then add DNA, mix well by flicking the tube about 12 times to create a vortexing action. Add Targefect-BAC next, mix well again by flicking the tube. Add Virofect last and mix again. Incubate the tubes at 37°C for 25 minutes to form the transfection complexes.
Add 0.25 ml of the transfection mix to 2 ml of complete media for 1 well for a 6-well dish (or for a 35 mm dish). Prepare 0.5 ml of transfection complex per 60 mm dish and 1 ml of transfection complex per 100mm dish. Swirl the dish to mix transfection complexes with the cell culture media (with 5-10% serum). Incubate overnight at 37° C in CO2 incubator. Replace the media with fresh complete media the next morning and assay at 36-48 hrs post-transfection.
Expected efficiency –30-40%, cells tested, Vero cells, 60% in HEK-293 cells. **Please do not use the Virofect enhancer when transfecting HEK-293 cells. For transfecting BAC DNA into HEK-293 cells use the Targefect-BAC reagent alone or the Targefect-293 reagent.**

This kit (catalog #BAC-01) contains sufficient Targefect reagent for performing 200 transfections in 12-well dishes or 100 transfections in 6-well dishes.

Product No.	Quantity	T.S. list Price
BAC-01	1	$350
BAC-10	10	$2500

Contents: **Targefect-BAC (0.6 ml) Virofect (0.6 ml), Peptide Enhancer (0.6 ml)**
Cell lines tested: **Vero, HEK-293, CV1, adipocytes, Hela and HUVECs**

PRODUCT CITATIONS USING TARGEFECT-BAC COMPONENTS FOR DELIVERY OF BAC DNA:

1.Gene amplification system based on double rolling-circle replication as a model for oncogene-type amplification
Takaaki Watanabe, Hideyuki Tanabe, and Takashi Horiuchi
Nucleic Acids Res., Sep 2011; 39: e106.
2. A sequence-independent *in vitro* transposon-based strategy for efficient cloning of genomes of large DNA viruses as bacterial artificial chromosomes
Fuchun Zhou, Qiuhua Li, and Shou-Jiang Gao
Nucleic Acids Res., Jan 2009; 37: e2.
3. Kaposi's Sarcoma-Associated Herpesvirus Latent Gene vFLIP Inhibits Viral Lytic Replication through NF-B-Mediated Suppression of the AP-1 Pathway: a Novel Mechanism of Virus Control of Latency Feng-Chun Ye, Fu-Chun Zhou, Jian-Ping Xie, Tao Kang, Whitney Greene, Kurt Kuhne, Xiu-Fen Lei, Qui-Hua Li, **and** Shou-Jiang Gao
J. Virol., May 2008; 82: 4235 - 4249.
4.Genetic disruption of KSHV major latent nuclear antigen LANA enhances viral lytic transcriptional program Qiuhua Lia, c, ,Fuchun Zhoua, b, Fengchun Yea, , Shou-Jiang Gaoa Virology, Volume 379, Issue 2, 30 September 2008, Pages 234-244
5. Kaposi's Sarcoma-Associated Herpesvirus Infection Promotes Invasion of Primary Human Umbilical Vein Endothelial Cells by Inducing Matrix Metalloproteinases
Li-Wu Qian, Jianping Xie, Fengchun Ye, and **Shou-Jiang Gao**

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PROTOCOL SHEET:

Store Virofect at -20°C or -70°C. Store the Targefect-BAC reagent and the Peptide Enhancer at 4°C. Do not vortex it and freeze it.

Transfection Protocols using Targefect-BAC

The Targefect-BAC transfection kit is a combination of the Targefect-BAC) with enhancer reagents for optimal transfection of BAC-DNA vectors (greater than 150 Kb). Two protocols are presented. One protocol uses the Targefect-BAC reagent in combination with the Peptide enhancer, an enhancer reagent that improves gene transfer by escorting genes to the nucleus. A major advantage of the Peptide enhancer protocol (see write up below) is that it uses significantly less BAC DNA than the second protocol which uses Targefect-BAC in combination with the Virofect enhancer. We suggest testing both protocols to see which works better with your cell type.

Please use antibiotic-free media for both protocols. Use serum-free media for complexing DNA with Targefect-BAC and enhancer.

VIROFECT ENHANCER PROTOCOL FOR BAC DNA TRANSFECTION

When transfecting BAC DNA in HEK-293 cells please do not use the Virofect enhancer ie follow the protocol below but omit the VIrofect enhancer

Set up cells to be transfected so that they are about 70% confluent at the time of the experiment.
Store Virofect at -20°C or -70°C. Store the Targefect-BAC reagent at 4°C. Do not vortex it and freeze it.
Use clear plastic tubes for complex formation.
Use serum-free DMEM (Dulbecco's modified eagle's medium containing 4500 mg/liter glucose). Make additions as follows
Prepare transfection complexes as follows:

Tube #1: To 0.5 ml of high glucose DMEM and 10ug of BAC DNA. Mix well by flicking the tube to create a vortexing action (do not vortex). Next add 25 ul of Targefect-BAC and mix well again.

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Tube 2: To 0.5 ml of high glucose DMEM and 6ug of BAC DNA. Mix well by flicking the tube to create a vortexing action (do not vortex). Next add 12.5 ul of Targefect-BAC and mix well again. Next add 25 ul of Virofect and mix well again.

Incubate the tubes at 37°C for 25 minutes to form the transfection complexes.

Add 0.25 ml of the transfection mix to 2 ml of complete media for 1 well for a 6-well dish (or for a 35 mm dish). Prepare 0.5 ml of transfection complex per 60 mm dish and 1 ml of transfection complex per 100mm dish. Swirl the dish to mix transfection complexes with the cell culture media (with 5-10% serum).

Incubate overnight at 37 º C in CO2 incubator. Replace the media with fresh complete media the next morning and assay at 36-48 hours post-transfection.

PEPTIDE ENHANCER PROTOCOL FOR BAC DNA TRANSFECTION

Preparation of cells:
Set up cells so they are approx.60- 70% confluent the day of the experiment.

Preparation of transfection complexes:
To 1 ml of serum-free DMEM add 1 ug DNA, 5 ul of Targefect-BAC (Targefect F-2) and 15 ul of the peptide enhancer. Mix well after each addition and incubate at 37 º C for 20 mins to form complexes.

Aspirate off all culture media form the cells to be transfected and wash cells once with serum-free DMEM.

Add 1 ml of transfection complex per well of a 6-well dish, Add 0.4 ml transfection complex per well of a 12-well dish or 0.2 mal transfection complex per well of a 24-well dish. Swirl the dish to make sure transfection complexes cover cells well. Incubate 2 hrs. at 37°C. Aspirate transfection complex after 2 hours and replace with appropriate volume of complete media i.e. media with serum (2 ml for a 6-well dish, 1 ml for a 12-well dish or 0.5 ml for a 24-well dish. If possible, we recommend using complete media that have 5 or 10% serum).

Assay at 24 hours post transfection. If cells need to go longer replace media the next day
Note: It is important to use antibiotic -free media. As it will reduces toxicity.

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FAST PROTOCOL FOR TRANSFECTION OF HEK-293 CELLS WITH BAC DNA (Targefect-BAC plus Peptide Enhancer)

- **Linearize BAC**
- **Purify the DNA with a phenol:chloroform extraction and Precipitation step**
- **Transfection mix: 1 ml serum free media (no antibiotics) 1 ug DNA, 5 ul Targefect-BAC, 15 ul protein enhancer, Mix well after each addition. Incubate at 37oC for 25 min**
- **Plate HEK293 500,00 cells per 6 well the night before**
- **Rinse the cells with serum free medium (SFM) before adding transfection mix**
- **Remove SFM and added transfection mix (1 ml) per well, 37oC for 2 hours**
- **Replace transfection mix with DMEM + 10% FBS + pen/strep**
- **Next day add the selection antibiotic**
- **Once the well became confluent (about 3 days), trypsinize cells and transfer to a 10 cm dish continuing selection.**
- **After one-week pick colonies**