TECHNICAL MANUAL

TransfectNow™ HEK293 Cells

Instructions for Use of Products
NC1001 and NC1002

Revised 9/23
TM690

Promega

TransfectNow™ HEK293 Cells

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

Transient transfection is a convenient approach for executing cell-based assays in easily transfectable cell lines such as HEK293 cells. For this reason, NanoBRET™ cell-based assay platforms from Promega make extensive use of the transient transfection approach. However, the requirement to maintain cells in continuous culture to support routine transfection can be tedious (1). Here we introduce TransfectNow™ HEK293 Cells[a,b] as a convenient and reliable alternative to using freshly cultured and harvested HEK293 cells.

Maintaining cells in continuous culture and then harvesting for use in an assay can have several disadvantages (1). Firstly, continuous culturing of cells requires technical expertise, time and expensive cell-culture consumables, including medium and sterile plasticware. Secondly, prior to performing transfections, cells must be grown and harvested at a certain cell density for the best transfection efficiency, requiring multiple days and potentially delaying experiments. Thirdly, if cells are cultured beyond a validated passage limit, their characteristics can change, which can be detrimental to downstream assay performance. These disadvantages can introduce day-to-day variability in assays and cause delays in performing the assays that rely on transient cell transfection.

Treating frozen, QC-tested thaw-and-use cells as reagents is an easy way to save time and reduce both day-to-day and user-to-user variability (2). This is especially helpful for workflows where many transfections are being performed simultaneously, such as for compound screening or target selectivity profiling experiments. For this purpose, we developed TransfectNow™ HEK293 Cells in a thaw-and-use format (Figure 1).

1. Description (continued)

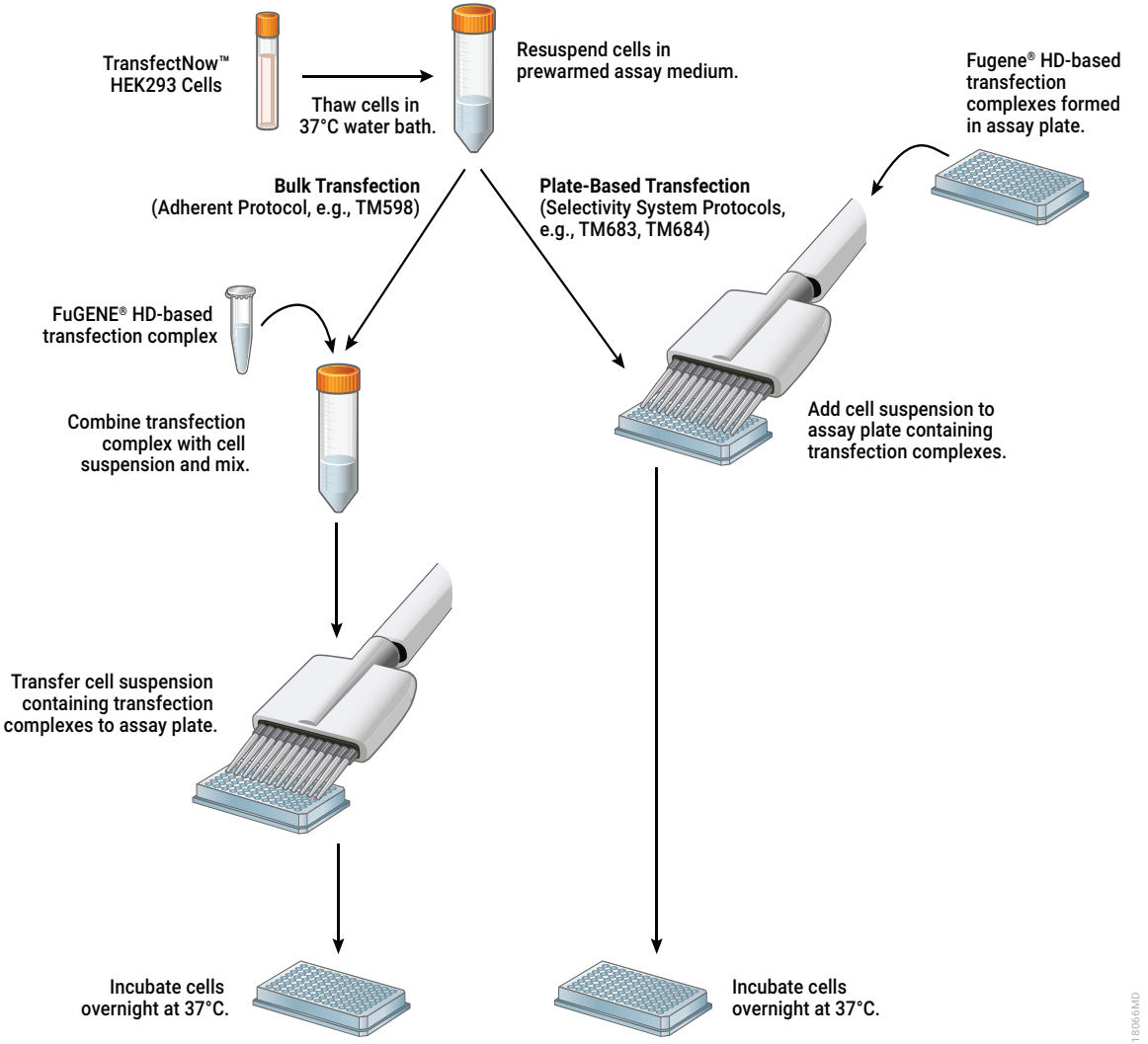

Figure 1. Example transfection workflow schematic using TransfectNow™ HEK293 cells, which can be used in both bulk transfection and plate-based transfection workflows.

TransfectNow™ HEK293 Cells performed equivalently to HEK293 cells from continuous culture, demonstrating both comparable transfection efficiency and assay window across 192 different kinase-NanoLuc® fusions (Figure 2). Though the TransfectNow™ HEK293 Cells were optimized for use in NanoBRET™ Target Engagement assays, they may also be compatible with other Promega assays within the NanoBRET™ platform (or other HEK293-based assays that require transfection) with additional development.

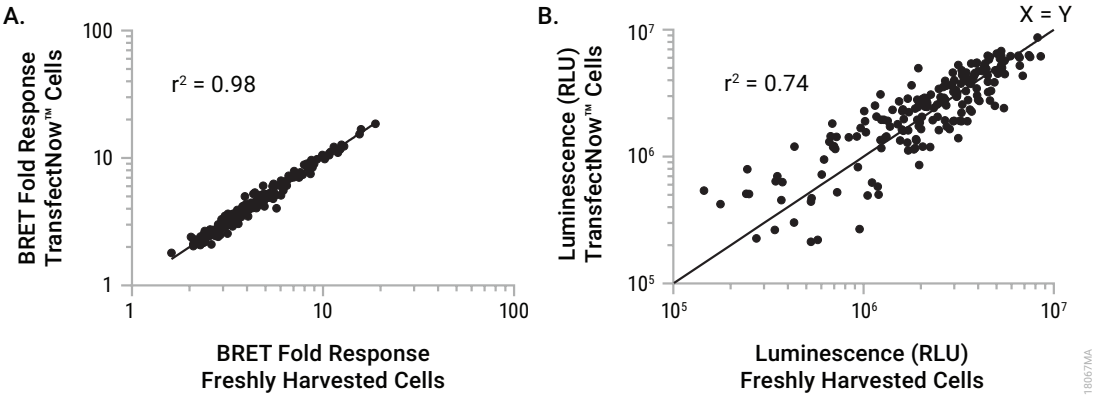

Figure 2. Performance comparison between freshly harvested and TransfectNow™ HEK293 Cells for NanoBRET™ TE Kinase Assays. Kinase-NanoLuc® fusions (192) were transfected into either freshly harvested HEK293 cells or TransfectNow™ HEK293 Cells using FuGENE® HD Reagent. Transfected cells were treated with NanoBRET™ Tracer K-10 and either a DMSO vehicle or a saturating dose of the CC1 pan-Kinase Inhibitor (Cat.# N2661) to compete with the BRET signal. After a 2-hour incubation, 3X Complete Nano-Glo® Substrate plus Extracellular NanoLuc® Inhibitor solution was added, and the luminescence and NanoBRET™ signals were measured using the NanoBRET™ 618 protocol on a GloMax® Discover System. **Panel A.** Comparable assay windows were observed between freshly harvested and TransfectNow™ HEK293 Cells. **Panel B.** Similar expression levels were also observed between freshly harvested and TransfectNow™ HEK293 Cells. Increased variation in expression at the lower end of the range is likely due to increased noise as expected from reductions in the signal-to-background ratio.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
TransfectNow™ HEK293 Cells, 1 × 0.5ml	**1 each**	**NC1001**

Includes:

- 1 vial TransfectNow™ HEK293 Cells, 0.5ml per vial

PRODUCT	SIZE	CAT.#
TransfectNow™ HEK293 Cells, 2 × 1ml	**1 each**	**NC1002**

Includes:

- 2 vials TransfectNow™ HEK293 Cells, 1ml per vial

Storage Conditions: Upon arrival, immediately transfer the cell vials to less than –140°C (freezer or liquid nitrogen vapor phase) for long-term storage. **Do not** store cell vials submerged in liquid nitrogen. Storing cells above –140°C will decrease cell viability and performance.

Cat.#	Contains sufficient cells for:
NC1001	2 × 96-well plates for adherent NanoBRET™ TE workflows, or
	1.25 × 384-well plates for adherent NanoBRET™ TE workflows
	2.5 × 96-well plates for NanoBRET™ TE K192 Kinase or CDK Selectivity Assays
NC1002	8 × 96-well plates for adherent NanoBRET™ TE workflows, or
	5 × 384-well plates for adherent NanoBRET™ TE workflows
	10 × 96-well plates for NanoBRET™ TE K192 Kinase or CDK Selectivity Assays

3. General Considerations

Please read through the entire protocol to become familiar with the components and assay procedure before beginning.

Remove the product label from the box containing vials with cells or note the catalog number and lot number from the label. This information can be used to download documents for the specified product from the web site, such as the Certificate of Analysis.

The TransfectNow™ HEK293 Cells are provided in frozen, thaw-and-use format and are ready to be used without any additional cell culture, propagation or cell counting. When thawed and diluted as instructed, the cells will be at the appropriate cell density for subsequent transfection. The cells are sensitive, and care should be taken to follow cell thawing and plating procedures exactly as described. Do not overmix or overwarm the cell reagents.

4. Cell Resuspension Protocol

Materials to Be Supplied by the User

- Opti-MEM™ I Reduced Serum Medium, no phenol red (Life Technologies, Cat.# 11058-021)
- fetal bovine serum (Hyclone Cat # SH30070.03, or Seradigm Cat # 1500-050)

Note: TransfectNow™ HEK293 Cells have been developed for use with NanoBRET™ Target Engagement assays and the assay medium described in Section 6.B is formulated for use in those assays. If alternate NanoBRET™ assay platforms are used with these cells, use the recommended assay media for your particular application and optimize accordingly.

4.A. Overview

TransfectNow™ HEK293 Cells can be used in place of freshly harvested HEK293 cells in NanoBRET™ Target Engagement System assays using an adherent protocol at the transfection step and can be used in both bulk transfection workflows and in place of freshly harvested HEK293 cells in plate-based transfection workflows. The *NanoBRET™ Target Engagement Intracellular Kinase Assay Adherent Format Technical Manual* #TM598, describes bulk transfection, while the *NanoBRET™ TE K192 Kinase Selectivity Systems* and *NanoBRET™ CDK Selectivity Systems Technical Manuals,* #TM683 and #TM684, respectively, describe plate-based transfection workflows. See Table 1 for details.

Instead of harvesting HEK293 cells from continuous culture, simply thaw TransfectNow™ HEK293 Cells, resuspend the cells in prewarmed assay medium and combine the cell suspension with the prepared transfection complex.

Notes:

a. TransfectNow™ HEK293 Cells are for single use only and should not be cultured or refrozen for additional use. Plan your experiments accordingly to optimize the cell suspension use.

b. In the context of most transfection workflows, we recommend first preparing the transfection complexes and then preparing the TransfectNow™ HEK293 Cell suspension while the transfection complexes are forming according to the instructions.

c. TransfectNow™ HEK293 Cells may also be compatible with other Promega cell-based assays within the NanoBRET™ platforms, using a modification of the cell resuspension procedures in Section 4.B. However, applications beyond NanoBRET™ TE have not been tested at this time.

Table 1. Resuspension of TransfectNow™ HEK293 Cells in Assay Medium.

	Bulk Transfection Formats		**Plate-Based Transfection Formats**	
	For protocols where cells and transfection complexes are mixed prior to seeding in assay plate.		For protocols where transfection complexes are formed in assay plate, then cells are added to plate.	
TransfectNow™ HEK293 Cells	0.5ml	1ml	0.5ml	1ml
assay medium	21ml	42ml	17ml	34ml
total volume	**21.5ml**	**43ml**	**17.5ml**	**35ml**
NanoBRET™ TE Protocols	TM598		TM683, TM684	

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4.B. TransfectNow™ HEK293 Cells Protocol

The TransfectNow™ HEK293 Cells are sensitive; follow the cell thawing procedures exactly as described. Do not overmix or overwarm the cell reagents. No additional cell culture or manipulation is required. We recommend that you thaw and dilute a maximum of two vials of TransfectNow™ HEK293 Cells at a time.

Follow the steps below using aseptic technique in a sterile cell culture hood.

1. Remove the appropriate number of vials of TransfectNow™ HEK293 Cells from storage at –140°C and transfer to the bench on dry ice.
2. Add the appropriate volume of prewarmed (37°C) assay medium (see Section 6.B) to a 50ml conical tube, depending on the size of vial you are thawing and the downstream assay you are performing. See Table 1 for cell resuspension volumes. If thawing multiple vials, scale volumes accordingly.
3. Warm the cells in a 37°C water bath until just thawed (approximately 1–2 minutes). While thawing, gently agitate and visually inspect.

Note: Do not submerge the vial completely. Do not invert the vial.

4. Immediately after the cells are thawed, dry the vial, then spray the vial with 70% ethanol.
5. Gently mix the cell suspension by pipetting, then transfer the cells to the 50ml conical tube containing assay medium. Mix well by gently pipetting or inverting 1–2 times.
6. The cell suspension is ready to be combined with the prepared transfection complex in the NanoBRET™ protocol of your choice. No cell counting or centrifugation steps are necessary post-thaw.

Note: See Section 6.C, Related Products, for a list of NanoBRET™ systems compatible with TransfectNow™ HEK293 Cells.

5. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
Weak expression levels (low RLU with 450nm band pass filter)	Insufficient cells per well can lead to low RLU. Handle and plate the cells according to the instructions to ensure a sufficient number of viable cells per well.
	Lack of serum in the assay medium. Include 1% FBS in the assay medium to increase transfection efficiency.

6. Appendix

6.A. References

1. Robinson, C.J. and Lamerdin, J. (2017) Ready-to-Use Cells in Bioassays for Quality Control of Biopharmaceuticals. Accessed 16-Feb-22. **https://www.bioanalysis-zone.com/ready-use-cells_spotlbioanalysis_of_biopharma/**
2. Arduengo, M. (2020) Maximize Your Time in the Lab: Improve Experimental Reproducibility with Thaw-and-Use Cells. Accessed: 2-Feb-22. **https://www.promegaconnections.com/thaw-and-use-cells/**

6.B. Composition of Buffers and Solutions

assay medium

99% Opti-MEM™-I Reduced Serum Medium, no phenol red
1% FBS

Prepare an appropriate amount of assay medium prior to transfection. Thaw the fetal bovine serum (FBS) in a 37°C water bath, taking care not to overheat it. Add 5ml of FBS to 500ml of Opti-MEM™ medium to yield 99% Opti-MEM™/1% FBS. Mix well and warm to 37°C prior to use.

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6.C. Related Products

NanoBRET™ TE Intracellular Kinase Assays

Product	Size	Cat.#
NanoBRET™ TE Intracellular Kinase Assay K-3	100 assays	N2600
NanoBRET™ TE Intracellular Kinase Assay K-4	100 assays	N2520
NanoBRET™ TE Intracellular Kinase Assay K-5	100 assays	N2500
NanoBRET™ TE Intracellular Kinase Assay K-8	100 assays	N2620
NanoBRET™ TE Intracellular Kinase Assay K-9	100 assays	N2630
NanoBRET™ TE Intracellular Kinase Assay K-10	100 assays	N2640
NanoBRET™ TE Intracellular Kinase Assay K-11	100 assays	N2650

Additional assay sizes are available. For a complete listing of the available kinase TE expression vectors and their NanoBRET™ tracer compatibility, see: **www.promega.com/kinasevectors**

NanoBRET™ TE CDK Selectivity Panels and Systems

Product	Size	Cat.#
NanoBRET™ TE CDK Vector Panel A	1 each	NP5000
NanoBRET™ TE CDK Vector Panel B	1 each	NP5100
NanoBRET™ TE CDK Selectivity System A	1 each	NP5050
NanoBRET™ TE CDK Selectivity System B	1 each	NP5150

NanoBRET™ TE K192 Selectivity Panels and Systems

Product	Size	Cat.#
NanoBRET™ TE K192 Kinase Vector Panel	1 each	NP4100
NanoBRET™ TE K192 Kinase Vector Panel, Small	1 each	NP4101
NanoBRET™ TE K192 Kinase Selectivity System	1 each	NP4050
NanoBRET™ TE K192 Kinase Selectivity System with Controls	1 each	NP4060

NanoBRET™ TE Nano-Glo® Substrate/Inhibitors

Product	Size	Cat.#
Intracellular TE Nano-Glo® Substrate/Inhibitor	100 assays	N2162
Intracellular TE Nano-Glo® Vivazine™ Inhibitor	1,000 assays	N2200

Additional sizes are available.

Transfection Reagents and Accessories

Product	Size	Cat.#
FuGENE® HD Transfection Reagent	1ml	E2311
Transfection Carrier DNA	5 × 20µg	E4881

Additional sizes are available.

Luminometers

Product	Size	Cat.#
GloMax® Discover System	1 each	GM3000

For Research Use Only. Not for Use in Diagnostic Procedures.

7. Summary of Changes

The following changes were made to the 9/23 revision of this document:

1. Added the NanoBRET™ TE K192 Kinase Selectivity and NanoBRET™ TE CDK Selectivity Systems to Figure 1, Table 1 and Sections 2, 4 and 6.

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[b]HEK293 cells were obtained under license from AdVec, Inc.

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