BQC TRANS

BQC TRANS / BQC TRANS PLUS / BQC TRANS ULTRA / BQC TRANS LT

(KT-05-001 / KT-05-002/ KT-05-003/ KT-05-004)

BioQuChem
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Introduction

Transfection allows the introduction of nucleic acids inside the cells.
It is currently used in a large number of experimental approaches like the introduction of foreign nucleic acids into cells to produce genetically modified cells.

**BQCtrans KT-05-001** Kit is an optimized formula for DNA transfection.

**BQCtrans PLUS KT-05-002** Kit is an optimized formula able to increase the efficiency of the transfection.

**BQCtrans ULTRA KT-05-003** Kit is an optimized formula able to minimize cell toxicity.

**BQCtrans LT KT-05-004** Kit is an optimized formula able to increase the efficiency of the transfection and to minimize cell toxicity.
**Materials**

Bioquochem TRANSFECTION KIT *KT-05-001, KT-05-003* contains:

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A</td>
<td>1</td>
<td>4°C</td>
</tr>
</tbody>
</table>

Bioquochem TRANSFECTION KIT *KT-05-002, KT-05-004* contains:

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A</td>
<td>1</td>
<td>4°C</td>
</tr>
<tr>
<td>Reagent Plus</td>
<td>1</td>
<td>4°C</td>
</tr>
</tbody>
</table>

➢ These kits are for R&D use only

⚠️ All these chemicals should be handled with care
**Assay Protocol**

**Short protocol:**

1. Seed cells until desired cell density in culture plates (6/12/24/48/96-well) or 60/100 mm plates

2. Let the reagents reach room temperature before use.

3. Add in a tube X µl of medium (medium + Reagent A from the next step) must be accordingly with column number 4 of the Table 1.

4. Add in the same tube X µl of Reagent A. Stir mixture. Not vortex.

5. Incubate for 5-15 minutes at RT or 37°C. Longer incubations may adversely affect transfections. This solution is called **diluted transfection reagent**.

6. For **KT-05-001** and **KT-05-003**: Add X µg of DNA to the diluted transfection reagent.

7. For **KT-05-002** and **KT-05-004**: Dilute DNA into Reagent Plus and then add X µg of DNA to the diluted transfection reagent.

8. Incubate for 10-60 minutes at 37°C.

9. Add transfection mixture (DNA+diluted transfection Reagent) into cell medium and shake

10. Visualize/ analyze the transfected cells after 1-7 days at 37°C.
**Assay Protocol**

**Detailed assay protocol:**

**A) Cell Growth**

Cell Growth can be done in media with or without serum/antibiotic. If you choose to perform the transfection in serum-free medium, replace the medium with serum-containing medium 4–12 hours after transfection.

**B) Preparing transfection mixture**

Perform this procedure immediately prior to transfection. Allow the reagents to stand at room temperature at least 10 minutes before use.

Then, an example for a 6 well plate and for a 3:1 Reagent A:DNA ratio is explained. Choose your Reagent A:DNA ratio and your adequate reagents volume following Annex I and II.

1. Transfer 97 μl of sterile medium (serum and antibiotic free) at room temperature to a polystyrene tube. The total volume (Medium + Reagent A from the next step) should be 100 μl. (See table 1 in Annex II).

2. Add 3 μl of Reagent A in the previous tube by pipetting directly into the serum-free medium previously added (the presence of serum at this stage is inhibitory). To mix, stir gently with the pipet tip. Do not vortex.

3. Incubate at room temperature or 37°C for 5-15 minutes. This mixture is called Diluted Transfection Reagent.

4. This step is different depending on the reference of the kit:
   - For KT-05-001/ KT-05-003: Add 1 μg of DNA (See table in Annex II) to the Diluted Transfection Reagent and mix gently with the pipet tip. Do not vortex. This is called Transfection Mixture.
   
   *Note: For an optimal transfection efficacy, avoid the DNA to contact with the tube*
Assay Protocol

-For KT-05-002/ KT-05-004: Dilute the DNA in the Reagent Plus (provided with BQC Transfection Kit) and incubate it for 5 minutes at 37°C. Then, add 1 μg of DNA (See table in Annex II) from this solution to the Diluted Transfection Reagent and mix gently with the pipet tip. Do not vortex. This is called Transfection Mixture.

Note: For an optimal transfection efficacy, avoid the DNA to contact with the tube

5. Incubate for 10-60 minutes at 37°C.

C) Transfection of the cells

6. Add the appropriate volume of the Transfection mixture (DNA+diluted transfection Reagent) (see Table 1 in Annex II) into cell medium and shake the plate back and forth to distribute the transfection mixture.

7. Incubate the plate in standard growth conditions until desired time (i.e.1-7 days) with or without replace of medium.
ANNEX I

Reagent A: DNA Ratio

The optimal Reagent A (μl): DNA (μg) ratio should allow the highest transfection efficiencies with the lowest level of toxicity. For most cell lines, Reagent A: DNA ratio is between 2:1 and 6:1.

These ratios are a good starting point for optimization experiments with your specific cell line and DNA. To optimize further, keep the quantity of DNA constant and increasingly add volumes of transfection reagents.

Excess DNA may inhibit transfection efficiency, so do not exceed a Reagent A: DNA ratio of 1:1.
ANNEX II

The transfection procedure, uses several sizes of culture dishes. The optimal ratio of Reagent A (μl): DNA (μg) must be determined for each plasmid and cell line, but ratios of 2:1 to 6:1 are recommended as starting points for optimization.

Table lists volume for generating Reagent A: DNA mixtures of a 3:1 ratio for commonly used tissue culture dishes. The volume of the transfection mixture may be scaled up by increasing the components proportionally to accommodate several transfections.

Table 1. Volume for generating Reagent A: DNA mixtures of a 3:1 ratio in different culture dishes

<table>
<thead>
<tr>
<th>Dish Format</th>
<th>Reagent A (μl)</th>
<th>DNA (μg)</th>
<th>Transfection Mixture/well (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>96 well</td>
<td>0.15</td>
<td>0.05</td>
<td>5</td>
</tr>
<tr>
<td>48 well</td>
<td>0.3</td>
<td>0.1</td>
<td>10</td>
</tr>
<tr>
<td>24 well</td>
<td>0.6</td>
<td>0.2</td>
<td>20</td>
</tr>
<tr>
<td>12 well</td>
<td>1.5</td>
<td>0.5</td>
<td>50</td>
</tr>
<tr>
<td>6 well</td>
<td>3.0</td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>60-mm</td>
<td>6.0</td>
<td>2.0</td>
<td>200</td>
</tr>
<tr>
<td>100-mm</td>
<td>18.0</td>
<td>6.0</td>
<td>600</td>
</tr>
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</table>
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Said refund or replacement is conditioned on buyer giving written notice to Bioquochem within 30 days after arrival of the material at its destination.

Expiration date: 1 year from the date of delivery

For further details, please refer to our website www.bqckit.com.