Protocol-at-a-Glance

TransFactor Chemiluminescent Kits
Protocol-at-a-Glance

(PT3757-2)

Please read the *User Manual* before using this Protocol-at-a-Glance. This abbreviated protocol is provided for your convenience, but is not intended for first-time users.

A. Prepare 1X TransFactor Buffer and Block Wells

1. Determine the amount of 1X TransFactor Buffer required:

   (No. assay strips) x 24.0 ml = Total Volume TransFactor Buffer

   Dilute the 10X TransFactor Buffer 1:10 with distilled water to obtain the above volume.

2. Prepare 1X TransFactor/Blocking Buffer:

1.8	ml/assay	1X TransFactor Buffer
0.06	g/assay	Blocking Reagent
1.8	ml/assay	Total Volume TransFactor/Blocking Buffer

Notes

- Mix the Blocking Reagent with the 1X TransFactor Buffer until the Blocking Reagent completely dissolves, then filter the TransFactor/Blocking Buffer through Whatman filter paper before use.
- Keep the remaining 1.2 ml/assay of 1X TransFactor Buffer to use as wash after the Secondary Antibody incubation.

B. Add Sample to TransFactor Well

1. Thaw the nuclear or whole cell extract slowly on ice.

   Note: After the nuclear or whole cell extract is thawed, we recommend centrifuging the sample at 20,000 g for 5 min at 4°C to remove residual cell debris. Including this step will decrease the variability of your results.

2. Proceed to Step 3 for designated* TransFactor kits or Step 4 for TransFactor Universal Kits.

3. **For designated* TransFactor Kits only:**

   a. Add 150 µl 1X TransFactor Blocking Buffer per well and incubate at room temperature for 15 min.

   b. Prepare sample by diluting the desired amount of cell extract to a final volume of 50 µl with 1X TransFactor/Blocking Buffer. Add the appropriate amount of Poly dIdC when required (see PT3594-3). Proceed to Step 5.

   Notes

   - Optimal extract concentration may vary depending on the transcription factor, cell type, and extraction method. To optimize the assay, perform a dose response curve with your cellular extract (see Figure 2 in the User Manual). We find that 5 µg of the provided Positive Control Cellular Extract usually works well as a starting point; however, some extracts perform better at lower concentrations.
   - For a background control, use 50 µl 1X TransFactor/Blocking Buffer alone.
   - Optimal Competitor Oligo concentration may vary depending on the transcription factor. For competition assays, add 1 µl Competitor Oligo (500 ng) to the sample and reduce the 1X TransFactor/Blocking Buffer volume sufficiently to maintain a total assay volume of 50 µl. If this does not generate an adequate decrease, then add more Competitor Oligo in subsequent competition assays.

4. **For TransFactor Universal Kits only:**

   a. Prepare sample by mixing the desired amounts of cell extract and Poly dIdC (see Notes below) with 2 pmol biotinylated oligo, and diluting the mixture to a final volume of 50 µl with 1X TransFactor/Blocking Buffer.

   Notes

   - Optimal extract concentration may vary depending on the transcription factor, cell type, and extraction method. To optimize the assay, perform a dose response curve with your cellular extract (see Figure 2 in the User Manual). We find that 5 µg of the provided Positive Control Cellular Extract usually works well as a starting point; however, some extracts perform better at lower concentrations.
   - For a background control, use 50 µl 1X TransFactor/Blocking Buffer alone.

*"Designated" refers to the Individual, Profiling, and Family Kits.

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- Optimal Competitor Oligo concentration may vary depending on the transcription factor. For competition assays, add 20 pmol Competitor Oligo to the sample and reduce the 1X TransFactor/Blocking Buffer volume sufficiently to maintain a total assay volume of 50 µl. If this does not generate an adequate decrease, then add more Competitor Oligo in subsequent competition assays.
- The biotinylated oligo can be a wild-type or mutant oligo.
- Optimal Poly dIdC concentration can vary with different transcription factors. We find that 0.5 µg of Poly dIdC per reaction is a good starting point.
- When multiple repeats of the same binding assay are desired, you can multiplex the same amount and mix in the same tube.

b. Incubate the samples on ice for 15 min.

c. Meanwhile, add 150 µl of 1X TransFactor/Blocking Buffer per well and incubate at room temperature for 15 min. Proceed to Step 5.

5. Remove the 1X TransFactor/Blocking Buffer.
6. Add the 50 µl sample to the well, and incubate for 60 min at room temperature.
7. Wash the wells 3 times with 150 µl 1X TransFactor/Blocking Buffer per well. Allow 4 min for each wash. After the final wash, remove 1X TransFactor/Blocking Buffer from the wells.

C. Incubate with Primary Antibody

1. Dilute Primary Antibody in 1X TransFactor/Blocking Buffer (see Data Sheet for required dilutions). Dilute sufficient Primary Antibody to yield 100 µl per assay well.
2. Add 100 µl of diluted Primary Antibody to each well. Incubate at room temperature for 60 min.
3. Wash the wells 3 times with 150 µl 1X TransFactor/Blocking Buffer per well. Allow 4 min for each wash. After the final wash, remove 1X TransFactor/Blocking Buffer from the wells.

D. Incubate with Secondary Antibody

1. Dilute Secondary Antibody in 1X TransFactor/Blocking Buffer (1:10,000 dilution forAnti-rabbit IgG-HRP and 1:2,000 dilution for Anti-mouse IgG-HRP). Refer to the Data Sheet to identify the correct Secondary Antibody for your assay.
2. Add 100 µl of diluted Secondary Antibody to each assay well and incubate at room temperature for 30 min.

   Note: Remove Chemiluminescent Substrates from the refrigerator and allow them to come to room temperature before use.
3. Wash the wells 4 times with 250 µl of **1X TransFactor Buffer** per well (no Blocking Reagent). Allow 4 min for each wash. After the final wash, remove 1X TransFactor Buffer from the wells.

   Note: The use of 1X TransFactor/Blocking Buffer for this wash increases background. Therefore, only use 1X TransFactor Buffer, without Blocking Reagent, after the Secondary Antibody incubation.

E. Chemiluminescent Signal Development

1. Calculate the total volume of chemiluminescent substrate by multiplying the number of wells in the assay by 100 µl/well.
2. Mix the appropriate volume of chemiluminescent substrate by mixing 1/2 volume Chemiluminescent Substrate A with 1/2 volume Chemiluminescent Substrate B.

   Note: Allow the Chemiluminescent Substrates to come to room temperature before use.
3. After mixing, allow the substrate mixture to incubate at room temperature for at least 2 min before adding it to the wells.
4. Add 100 µl of mixed substrate to each well.
5. Measure chemiluminescent intensities with a luminometer. For highest sensitivity, read the signals immediately after adding the substrate.

   Notes

   • The signals are stable within 10 min (See Figure 8 in Section VIII of the User Manual).

   • We recommend the Monolight™ 3096 Microplate Luminometer (Cat. No. 551280).