

BD Mercury™ TransFactor Profiling Kit— Oncogenesis 3

A high-throughput assay for detecting DNA-protein interactions

- Analyze the DNA-binding activity of multiple transcription factors simultaneously with one assay
- Faster and more sensitive than gel-shift assays
- Flexible 96-well format

A key step in the regulation of gene expression is the binding of a transcription factor to its *cis*-acting DNA response element. In the past, researchers routinely measured such activities using an electrophoretic mobility gel-shift assay (EMSA). Today, however, many are discovering that DNA-protein interactions can be measured with greater sensitivity and in shorter time using non-radioactive, 96-well plate assays developed by BD Biosciences Clontech (1, 2).

BD Mercury™ TransFactor Profiling Kits† are the new high-throughput alternative to EMSA and supershift assays. These kits provide a highly specific immunoassay for detecting and quantifying the DNA-binding of several transcription factors involved in inflammation and oncogenesis (3, 4). Our newest kit, **Oncogenesis 3**, lets you measure HIF-1 α , HIF-1 β , Egr-1, c/EBP, Oct I, and Oct II, adding six more entries to the long list of factors you can now profile with our ready-to-use kits (Table I).

TransFactor Profiling Kits are ideal for studying transcriptional regulation in different cell lines and tissues, and for investigating potential drug targets. Each kit contains a 96-well plate (Figure 1) for measuring the DNA-binding behavior of six different transcription factors. Individual wells have been precoated with the DNA consensus binding sequence for a specific factor. To perform an assay, add nuclear extract from mammalian cells to the wells and incubate to allow the transcription factor to bind its sequence. Wash away the unbound proteins, and add primary antibody. Then, add HRP-conjugated secondary antibody, incubate with HRP substrate, and measure the color intensity.



Figure 1. TransFactor Kits are available in individual and profiling formats. Individual Kits let you investigate a single transcription factor in depth. Profiling Kits, on the other hand, enable you to screen the DNA-binding activities of multiple factors involved in specific biological processes such as inflammation and oncogenesis. Profiling plates (shown above) are divided into six sets of color-coded wells; each set contains the *cis*-acting DNA element for a specific transcription factor. All TransFactor Plates consist of unique snap-off wells, so you can reconfigure the plates to fit your experimental design. You may perform all 96 reactions at once, or remove wells (individually or in strips) for use at a later time.

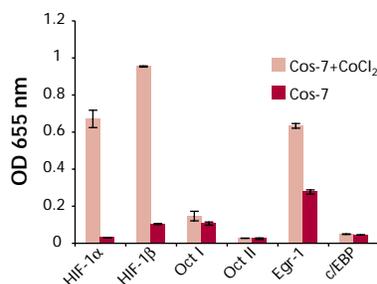


Figure 2. Transcription Factor Profiling with Oncogenesis 3. Cos-7 cells were treated with 0.15 mM CoCl₂ for 23 hr. Nuclear extracts were then prepared using the BD TransFactor Extraction Kit (#K2064-1), and assayed according to the protocol in the BD Mercury TransFactor Kits User Manual (PT3594-1).

TransFactor assays typically take 3–4 hours, and are 10 times more sensitive than EMSA (1, 2). The flexible 96-well format gives you the ability to compare multiple samples simultaneously (Figure 2). You can even perform competition assays to assess binding specificity and to determine the key bases in the protein-binding DNA consensus sequence.

BD Mercury™ TransFactor Profiling Kits

Size	Cat. #
Profiling Kit—Oncogenesis 1 96 rxns	K2073-1
Profiling Kit—Oncogenesis 2 96 rxns	K2075-1
Profiling Kit—Oncogenesis 3 96 rxns	K2076-1
Profiling Kit—Inflammation 1 96 rxns	K2062-1
Profiling Kit—Inflammation 2 96 rxns	K2072-1

BD Mercury™ Individual TransFactor Kits

Size	Cat. #
NF κ B p50 Kit 96 rxns	K2058-1
STAT1 Kit 96 rxns	K2059-1
c-Jun Kit 96 rxns	K2061-1
c-Fos Kit 96 rxns	K2065-1
CREB-1 Kit 96 rxns	K2066-1
NF κ B p65 Kit 96 rxns	K2067-1
Rb Kit 96 rxns	K2068-1
DP-1 Kit 96 rxns	K2069-1

Related Product

- TransFactor Extraction Kit (#K2064-1)

References

1. BD Mercury TransFactor Kits (January 2002) *Clontechiques XVII*(1):8–9.
2. Shen, Z., *et al.* (2002) *Biotechniques* **32**:1168–1177.
3. Two New BD Mercury TransFactor Profiling Kits (April 2002) *Clontechiques XVII*(2):20.
4. BD Mercury TransFactor Profiling Kit—Oncogenesis 2 (July 2002) *Clontechiques XVII*(3):15.

† Patent Pending

Table I: Transcription factors profiled by BD Mercury™ TransFactor Kits

Oncogenesis 1

DP-1, E2F-1, Rb, p107, E2F-2, Sp-1

Oncogenesis 2

c-Myb, c-Myc, Max, USF1, USF2, p53

Oncogenesis 3

HIF-1 α , HIF-1 β , Egr-1, c/EBP, Oct I, Oct II

Inflammation 1

NF κ B p50, NF κ B p65, c-Rel, ATF2, CREB-1, c-Fos

Inflammation 2

c-Jun, c-Fos, FosB, JunD, Sp-1, STAT1