EpiQuik™ General Protein-DNA Binding Assay Kit (Fluorometric)

Base Catalog # P-2005

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik*™ General Protein-DNA Binding Assay Kit (Fluorometric) is suitable for analyzing protein-DNA interactions *in vitro*, specifically for detecting transcription factor activation using mammalian tissue and cell extracts.

The *EpiQuik*™ General Protein-DNA Binding Assay Kit (Fluorometric) offers a flexible choice of ologonucleotides and antibodies. Like using other protein-binding assay kits, if you use the *EpiQuik*™ General Protein-DNA Binding Assay Kit (Fluorometric), choice of a good antibody is required for capturing the protein/DNA complexes.

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com

KIT CONTENTS

Components	96 assays P-2005-96
PF1 (10X Wash Buffer)	20 ml
PF2 (Antibody Buffer)	6 ml
PF3 (Extract Cleaner)*	100 µl
PF4 (Assay Buffer)	10 ml
PF5 (Oligo Release Solution)	11 ml
PF6 (Fluoro Developer)*	0.2 ml
DTT (500X)*	22 µl
96-Well Assay Plate	1
8-Well Assay Strips (with Frame)	12

** For maximum recovery of the products, centrifuge the original vial after thawing prior to opening the cap.*

SHIPPING & STORAGE

The kit is shipped in two parts: the first part at ambient room temperature and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store **PF3** and **PF6** at –20°C; (2) Store, **PF1**, **DTT** and **8-Well Assay Strips** at 4°C; (3) Store **all other components** at room temperature. All components of the kit are stable for 6 months from the date of shipment, when stored properly.

*Note: Check if wash buffer, **PF1**, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved.*

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Variable temperature waterbath
- ☐ Vortex mixer
- ☐ Fluorometer/Fluorescence Microplate Reader
- ☐ Desktop centrifuge (up to 14,000 rpm)
- ☐ Orbital shaker
- ☐ Pipettes and pipette tips
- ☐ 1.5 ml microcentrifuge tubes
- ☐ Oligonucleotide of interest
- ☐ Primary antibody of interest

GENERAL PRODUCT INFORMATION

Quality Control: EpigenTek guarantees the performance of all products in the manner described in our product instructions.

Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

Usage Limitation: The *EpiQuik*™ kits are for research use only and are not intended for diagnostic or therapeutic application.

Intellectual Property: *EpiQuik*™ is a trademark of EpigenTek Group Inc.

A BRIEF OVERVIEW

Protein-DNA interaction plays a critical role in cellular functions such as signal transduction, gene transcription, chromosome segregation, DNA replication and recombination, as well as epigenetic silencing. Identifying the genetic targets of DNA binding proteins and knowing the mechanisms of protein-DNA interaction is important for understanding cellular processes.

Measurement of direct interactions between protein and DNA *in vitro* has an advantage for analyzing binding of different transcription factors to specific DNA consensus sequences located in gene promoters. Several methods such as electrophoretic mobility shift assay (EMSA) and reporter gene assay have been developed to analyze direct interactions between protein and DNA *in vitro*. However, these methods available so far are time consuming, labor intensive, and have low throughput, or generate radioactive waste.

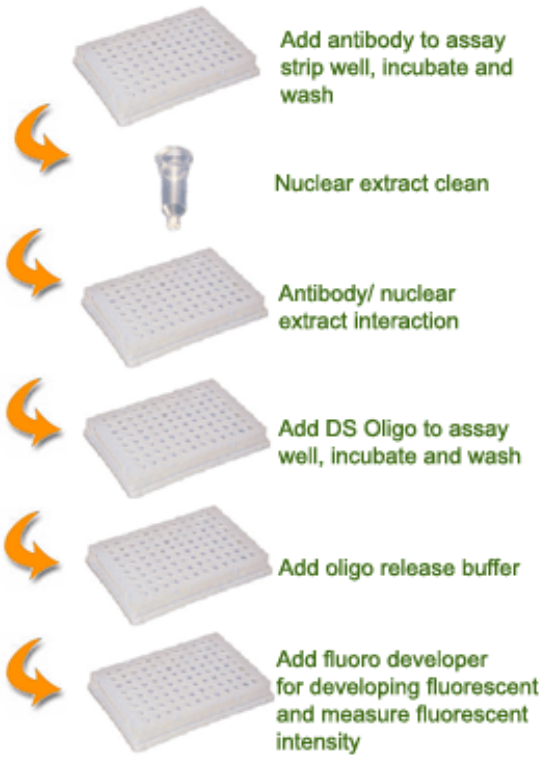
The *EpiQuik*™ General Protein-DNA Binding Assay Kit (Fluorometric) uses a unique procedure and composition to investigate protein-DNA interaction *in vitro* efficiently. This kit has the following features:

- The fastest procedure: a complete assay can be finished within 3 hours.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- Fluorometrically qualify or quantify protein activation and use radioactive-free materials: safer to handle.
- No requirement for coating antibodies, using secondary antibodies, or for labeling oligo-nucleotides: save time and reduce labor.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The *EpiQuik*™ General Protein-DNA Binding Assay Kit (Fluorometric) is designed for measuring the transcription factor (TF) of DNA binding activity in nuclear extracts. In this assay, target TF in

nuclear extract is captured by high affinity antibodies onto the assay microwells. A double-stranded oligonucleotide (oligo), containing DNA binding consensus sequence for the target TF, is added into the microwell and bound to active TF in the binding assay buffer. The bound oligo is then fluorometrically detected.



Schematic Procedure for Using the *EpiQuik*™ General Protein-DNA Binding Assay Kit (Fluorometric)

PROTOCOL

1. Prepare nuclear extracts from treated or untreated cells/tissues by using your own successful method. For your convenience and the best results, EpigenTek offers a nuclear extraction kit (Cat. No. OP-0002-1) optimized for use with the *EpiQuik*™ series.

2. Determine the number of strip wells required. Leave these strips in the plate frame (remaining unused strips can be placed back in the bag. Seal the bag tightly and store at 4°C). Dilute **PF1** with distilled water (pH 7.2-7.5) at a 1:10 ratio (e.g., 1 ml of **PF1** + 9 ml of distilled water). Wash the strip wells once with 150 µl of the **diluted PF1**.

3. Dilute your desired *primary antibody* (IgG type, 1:100) to 10 µg/ml with **PF2**. Add 50 µl of the *diluted primary antibody* to the strip wells, cover the wells with Parafilm M, and incubate at room temperature for 1-1.5 hours. Meanwhile, proceed to step 4.

4. Clean nuclear extracts by adding **PF3** at a 1:10 ratio to the nuclear extract and incubate for 5 minutes on ice. Centrifuge at 12,000 rpm for 2 minutes at 4°C to collect supernatant (*cleaned nuclear extracts*).

5. Aspirate antibody solution and wash each well with 150 µl of **diluted PF1** three times.

6. Add 100 µl of **diluted PF1** into each well, followed by adding 4-6 µl of *cleaned nuclear extracts* (5-20 µg) or purified transcription factor proteins (0.5-1 µg). Incubate at room temperature for 1 hour on a rocking platform (50-100 rpm). For the blank, add 4-6 µl of **diluted PF1** instead of nuclear extracts.

7. Aspirate the solution and wash each well with 150 µl of **diluted PF1** three times.

8. Prepare the **Complete PF4 Solution** by adding 1 µl of **DTT** (500X) to 500 µl of **PF4**. Add 50 µl of the **Complete PF4 Solution** and 2 µl (150-200 ng) of your *double-stranded oligonucleotide (DS Oligo)* to the wells. Mix and incubate at 37°C for 1 hour.

9. Aspirate and wash each well with 150 µl of **diluted PF1** five times.

10. Add 100 µl of **PF5** Oligo Release Solution to each well and incubate at 55°C for 15 minutes.

11. Transfer the solution to the 96-well assay plate provided. Add 2 µl of **PF6** to each well and measure fluorescence on a fluorometer at Ex 495 nm and Em 520 nm.

$$\text{Binding activity} = \text{RFU (sample} - \text{blank)} \times \text{sample dilution}$$

TROUBLESHOOTING

No Signal for the Sample

The protein sample is not properly extracted.	Ensure the protein extraction protocol is suitable for nuclear protein extraction.
The protein amount is added into the well insufficiently.	Ensure extract contains a sufficient amount of proteins.
The sample is not prepared from fresh cells or tissues.	The nuclear extracts from frozen cells or tissues significantly lose binding activity. A fresh sample should be used.
Nuclear extracts are incorrectly stored or have been stored for a long period of time.	Ensure the nuclear extracts are stored at –80°C for no more than 8 weeks.

High Background Present for the Blank

The well is not washed sufficiently.	Check if wash at each step is performed according to the protocol.
Nuclear extract is not sufficiently cleaned.	Ensure PF3 is added and incubated for a sufficient amount of time, as stated in step 4.
Insufficient antibody dilution.	Increase antibody dilution.

RELATED PRODUCTS

P-2004 *EpiQuik*™ General Protein-DNA Binding Assay Kit (Colorimetric)