

Marligen's Multiplex Transcription Factor Testing Services

The process is easy. Place your order, send us your cells or tissues, and we will send you your results. Testing services Include:

- Sample Preparation
- Sample Testing
- Data Analysis

Ordering Information:

Marligen will prepare nuclear extracts from your cells or tissues, test the extracts using our multiplex transcription factor profiling technology and summarize the results for you. We offer you a choice of two transcription factor profiling panels described below. We highly recommend that Marligen prepare the nuclear extracts from your samples to ensure optimal results. If you choose to prepare your own nuclear extracts, it is important that you consult with our Technical Services Department in advance, as not all reagents and methods are compatible with the subsequent assays.

| Catalog No.* | Assay Description | Transcription Factor Binding Sites |
|--------------|--|---|
| 11907-003 | Multiplex Transcription Factor Testing Service (20-plex) | AP-1, AP2, AR, CRE-ATF, CREB, E2F1-5, E2F6, EGR, GATA, HNF-1, HNF-4, ISRE, Myc-Max, NF-1, NFκB, p53, PPAR, SMAD 2/3, SP1 and YY1 |
| 11907-001 | Multiplex Transcription Factor Testing Service (50-plex) | AP-1, AP2, AML-1, AP4, AR, CRE-ATF, C/EBPα, C/EBPβ, C/EBPγ, CREB, E2F1-5, E2F6, EGR, ER, ETS, GATA, HFH-3, HIC-1, HIF, HNF-1, HNF-3, HNF-4, HSF-1, ISRE, MEF-2, MTF1, c-Myb, Myc-Max, NFAT, NF-1, NFκB, NFY1, Octamer, p53, PAX6, PBX, PLZF, PPAR, SAF-1, SOX9, STAT, SREBP, SMAD1/5, SMAD 2/3, SP1, SRE, TGIF, TAL1, TCF/LEF and YY1 |
| 11907-002 | Nuclear Extract Preparation | NA |

**A minimum of ten samples required per project.*

For pricing inquiries, to obtain quotations or to place your order, please contact our customer service representatives by telephone at 866-464-4990 or by e-mail at customer.service@marligen.com.

Once you have placed your order, we strongly recommend that you consult with one of our research and development scientists prior to performing your experiment to discuss sample preparation, handling and shipping.

Please contact Technical Support at technical.support@marligen.com if you are interested in sites that do not appear on the menu of the multiplex panel. We are continually expanding the selection of sites available, and we offer custom development services for new sites of particular interest to investigators.



Shipping Instructions

Please read and follow these instructions carefully. **Maintain samples at -80°C at all times.**

Clearly label samples and place them in a plastic bag along with a description of the samples (cell, tissue, or extract type). Pack your samples on at least 6 KG of dry ice in a Styrofoam cooler with an outer cardboard container. Be sure to label the outer container with “Perishable” and “Dry Ice” labels.

The address to use for your shipment is:

Marligen Biosciences Inc.
2502 Urbana Pike
Ijamsville, MD 21754
Attn: Testing Services Manager
Tel 301-874-4990-Ext 111

We recommend that you use standard overnight Federal Express for deliveries. Shipments should be sent on **Monday, Tuesday or Wednesday** only. It is important that you notify Marligen’s Customer Service Department via email at customer.service@marligen.com when you are sending samples for testing. Please include the name of the carrier, the tracking number and the expected delivery date for your shipment to arrive at Marligen so we can track the shipment and make sure it arrives in good condition. We will notify you when the samples have been received and the contents of the shipment have been verified against your packing slip.

[For international customers, please contact your local distributor for their guidelines for sending your samples to Marligen Biosciences.]

Turnaround Time and Results

Upon receipt, your samples will be stored frozen until they are processed. Unless otherwise requested, the multiplexed transcription factor assay will be performed in triplicate for each sample. A report summarizing the results will be prepared that includes the raw mean fluorescence intensities (MFIs) as an Excel file, graphs and a brief summary of the data. Typically an electronic copy of a report summarizing the results is transmitted within 10 business days after receipt of the samples.

Please contact our testing services technical staff at tfservices@marligen.com if you are interested in obtaining an example of the results report.



Sample Preparation Guidelines

Marligen's Multiplex Transcription Factor Assays have been validated on a wide range of samples derived from different cell lines, tissues, and species including human, mouse, and rat.

Amount of Sample Required:

Yields of protein from cells and tissues can vary greatly. It is recommended for a first experiment that you provide at least 25 mgs of tissue or 10 million cells for each sample to be tested to ensure that there is sufficient material to generate reliable results. If samples are limited and customers may want to consider a pilot experiment to verify that sufficient material is available in their samples for performing the testing. Our technical experts will discuss this with you before you begin. They can be reached at tfservices@marligen.com or at (301)-874-4990 ext 111. After this pilot experiment, our technical staff will make recommendations as to the amount of cells needed to perform additional multiplex assays using the same cells or tissues.

Protocol for Preparing Tissues or Cells

- Tissues –Tissue samples should be snap frozen, stored at -80°C until shipping on dry ice. If you have less than 25 mgs of tissue, please contact our technical staff at tfservices@marligen.com.
- Suspension cells – Centrifuge gently ($<1500\text{g}$) to pellet cells and remove media. Resuspend cells in 1ml of cold PBS. Transfer to microfuge tubes and pellet at 3000 rpm in a microcentrifuge (Eppendorf model 5415G or equivalent). Completely remove PBS from cells, snap freeze the pellets and store at -80°C until shipping on dry ice.
- Adherent cells – Prior to freezing and shipping your samples, process adherent cells using Marligen's **Adherent Cell Lysis Solution** (Catalog No. 11984-050) and the protocol supplied below.

Protocol for Preparing Nuclear Extracts

We recommend that Marligen prepare the nuclear extracts from your samples for the testing services.

If you wish to prepare your own samples, we strongly recommended that extracts be prepared using Marligen's **Nuclear Extraction Kit** (Catalog No. 11906-100) and cannot guarantee satisfactory results will be obtained with other reagents or methods. Please consult Marligen's technical staff if you are considering other kits or protocols for preparing samples.

We recommend that you provide 20-30 μg of extracts at a concentration of 2-10 $\mu\text{g}/\mu\text{l}$ to ensure adequate sample is available for optimal results with testing in triplicate.

Freeze extracts at -80°C immediately after they have been prepared.



Protocol for Preparing Adherent Cells for Nuclear Extraction

This protocol outlines the method used to harvest adherent cells prior to taking nuclear extracts. It requires Adherent Cell Lysis Solution (Cat. # 11984-050.)

1. Remove cell culture media by aspiration and wash cells twice with 2 – 10 ml of ice-cold PBS (not containing calcium or magnesium).
2. Calculate the amount of Adherent Cell Lysis Solution required using the chart below as a guide.

| Culture Vessel | Volume of Adherent Cell Lysis Solution |
|--------------------|--|
| 6-well plate | 500 µl/well |
| 100mm X 20 mm Dish | 2 ml/dish |
| T-25 Flask | 500 µl/flask |
| T-75 Flask | 2 ml/flask |
| T-175 Flask | 4 ml/flask |

3. Prepare Complete Adherent Cell Lysis Solution in a pre-chilled container according to the Table below.

| Add the following for each 1ml of Adherent Cell Lysis Solution | µl to add | Final Concentration |
|--|-----------|---------------------|
| Protease Inhibitor (Sigma Cat.# P8340) | 10 | 1x |
| *Ser/Thr Phosphatase Inhibitor (Sigma Cat.# P2850) | 10 | 1x |
| *Tyrosine Phosphatase Inhibitor (Sigma Cat.# P5726) | 10 | 1x |
| 100mM DTT (Sigma Cat.# D9779) | 10 | 1mM |
| 10mM PMSF (Sigma Cat.# P7626) | 10 | 0.1mM |

**Phosphatase inhibitors are recommended to preserve transcription factor activity, but may be omitted depending on the individual applications.*

4. Add Complete Adherent Cell Lysis Solution to cover cells. Collect cells with a rubber policeman or cell scraper and transfer to a 15 ml conical tube.
5. Pellet the cells by centrifugation at 800 x g for 5 minutes at 4° C and remove the supernatant.
6. Freeze cells at –80° C and ship samples on dry ice.