Procarta™ Transcription Factor Assay Kit

User Manual

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Panomics

Panomics, Inc.

Procarta Transcription Factor Assay Kit User Manual

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When describing a procedure for publication using this product, we would appreciate it if you would refer to it as the Procarta™ Transcription Factor Assay Kit.

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Contents

About the User Manual

Who Should Read this Manual

Anyone that has purchased a Procarta Transcription Factor (TF) Assay Kit from Panomics to perform profiling of up to 40 different transcription factors per reaction in the following sample types:

- Nuclear extracts from cultured cells
- Cell lysates from cultured cells

What this Manual Covers

This manual provides recommendations and step-by-step procedures for the following:

- Guidelines for assay design and data analysis
- Sample and assay preparation
- Set up and operation of the vacuum manifold system
- Assay procedure
- Troubleshooting

Safety Warnings and Precautions

CAUTION All chemicals should be considered potentially hazardous. We recommend that this product and its components be handled by those trained in laboratory techniques and be used according to the principles of good laboratory practice.

CAUTION This kit contains small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. When disposing, flush drains with a large volume of water to prevent azide accumulation. Observe all state and local regulations for disposal.

Note This product is intended for research use only. Not for diagnosis of disease in humans or animals.

For More Information

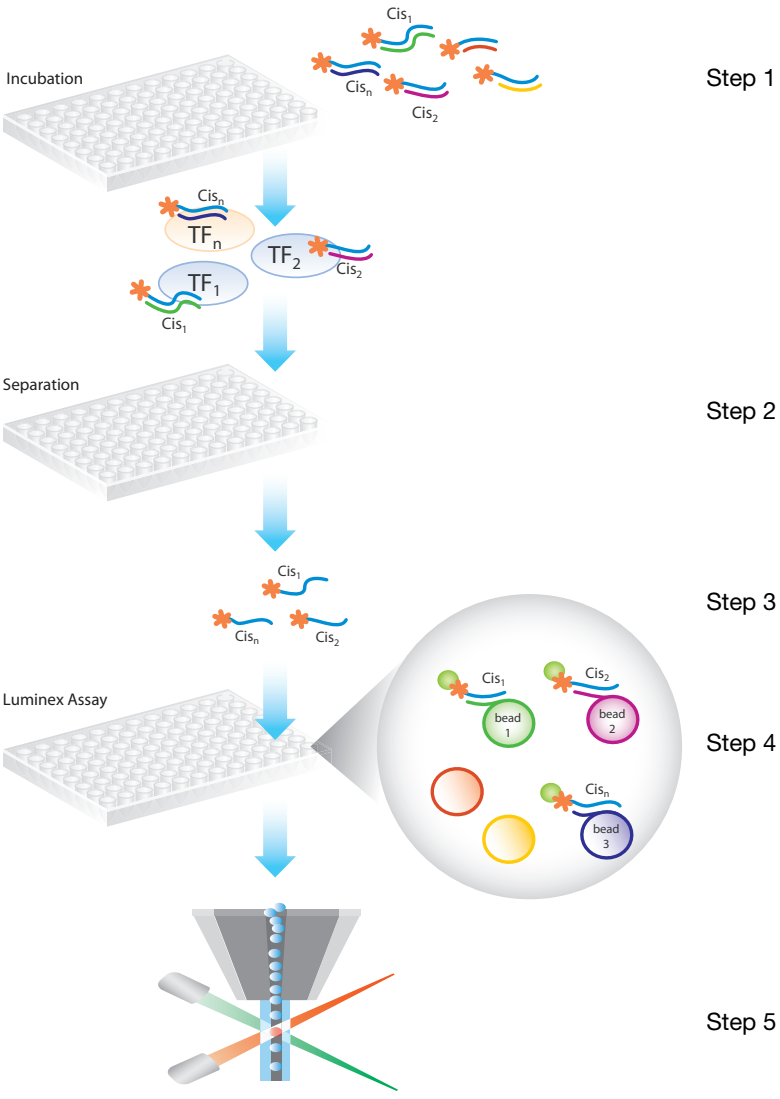
For information about the Procarta products mentioned in this manual, visit our website at www.panomics.com.

About the Procarta Transcription Factor Assay Kit

Fundamentals of Procarta Transcription Factor Assay

The Procarta TF assays combine two technologies: a patent-pending detection probe separation method and xMAP® (multi-analyte profiling beads). Together, these two technologies enable quantitative measurement of DNA binding activity of up to 40 transcription factors from as little as 500 ng of protein from a whole-cell lysate or 250 ng of protein from a nuclear extract. The xMAP technology, developed by Luminex Corp., combines flow cytometry, fluorescent-dyed microspheres (beads), lasers, and digital signal processing to effectively allow multiplexing of up to 100 unique assays within a single sample.

Assay Overview



Assay overview:

Step	Action
1	Incubate nuclear extract or cell lysate with biotin-labeled DNA binding probes to form protein/DNA complexes.
2	Separate protein/DNA complexes from unbound probes with a separation plate.

Assay overview: *(continued)*

Step	Action
3	Elute TF-bound detection probes from the separation plate.
4	Denature and hybridize eluted detection probes with TF-specific antisense conjugated beads.
5	Detect Streptavidin-conjugated R-phycoerythrin (SAPE) probe-bound beads with a Luminex instrument.

Available Kit Formats

Procarta TF Assay Kits are available in ¼- and 1-plate 96-well formats for the standard 40-plex. Assay kits contain all the reagents required to detect transcription factors from prepared whole-cell lysates or nuclear extracts, including nuclear extract and whole-cell lysate control samples. Kits for the preparation of whole cell lysates or nuclear extracts are sold separately.

Additional Procarta TF Assay Kit are available as:

- User selected (3–39 plex) assay kits in 1-plate formats. Selected TFs are provided in premixed, ready to use format. All assay kits include NFκB in order to run the positive and negative controls provided in the kit.

Procarta Transcription Factor Assay Kit Contents and Handling Conditions

Kit Contents and Storage

The Procarta TF Assay Kit contains the following components. Refer to the product insert for quantities and details of components supplied.

Procarta Transcription Factor Assay Kit components:

Component	Description	Storage
Positive Control Nuclear Extract	Untreated HeLa nuclear extract with addition of NFκB recombinant protein	–80 °C
Negative Control Nuclear Extract	Untreated HeLa nuclear extract	–80 °C
Positive Control Whole Cell Lysate	Untreated HeLa Whole Cell Lysate with addition of NFκB Recombinant Protein	–80 °C
Negative Control Whole Cell Lysate	Untreated HeLa Whole Cell Lysate	–80 °C
Detection Probes, premixed	Biotin-labeled DNA binding consensus sequences for specific TFs	–20 °C
Sample Dilution Buffer	Aqueous buffered solution for dilution of nuclear extract or whole cell lysate samples	–20 °C
DTT, 100 mM	Aqueous solution for stabilizing protein	–20 °C
Protease Inhibitor Cocktail	Aqueous solution for inhibiting protease activity	–20 °C
Binding Buffer	Aqueous buffered solution for protein/DNA binding	2–8 °C

Procarta Transcription Factor Assay Kit components: *(continued)*

Component	Description	Storage
Separation Buffer	Aqueous buffered solution for separation of protein/DNA complex from free probes	2–8 °C
Assay Buffer	Aqueous buffered solution for hybridization	2–8 °C
Wash Buffer	Aqueous buffered solution for Capture Bead washing	2–8 °C
Reading Buffer	Aqueous buffered solution for detection of Capture Beads	2–8 °C
Capture Beads, premixed	Pre-mixed beads conjugated with oligo corresponding to the Detection Probes	2–8 °C/Dark
Streptavidin-PE	Streptavidin-conjugated R-Phycoerythrin for detection of bound biotin probes	2–8 °C/Dark
Elution Buffer	Aqueous buffered solution for eluting the bound TF probes from protein	15–30 °C
PCR Plate	96-well clear PCR plate for Protein/DNA Complex formation	15–30 °C
Sample Collection Plate	96-well clear skirted-PCR plate	15–30 °C
Utility Plate	96-well clear polystyrene plate for collection of waste during the separation of TF-Bound Detection Probes. Also used as a holder for the Filter Plate during the detection of eluted Detection Probes	15–30 °C
Separation Plate	96-well white plate for the separation of Protein/DNA complexes from free probes	15–30 °C
PCR Plate Seal	Plastic sealer for sample collection plate used to denature samples at 95 °C and other PCR plates	15–30 °C
Plate Seal	Adhesive-backed foil for sealing filter plates during bead assay	15–30 °C
Filter Plate	96-well sterile filter plate	15–30 °C

Kit Handling

- Store all controls at -80°C, avoid multiple freeze/thaws.
- If precipitates occur in the Elution Buffer, warm to 37 °C with gentle swirling.

Required Materials and Equipment Not Provided

Equipment

Item	Source
Vacuum filtration system	Millipore (P/N MAVM0960R and WP6111560)
PCR Instrument for controlled incubation of samples in PCR Plates	MJ Research Model PCT-200 or equivalent
Microplate centrifuge capable of working at 4 °C	Eppendorf 5415D or equivalent

Item	Source
Microplate shaker	Labline model 4625 or equivalent with 3 mm orbit
Shaking incubator with microplate adaptor	E&K Scientific (Vortemp 56 P/N S-2056) or equivalent
Luminex or Luminex-based instrument	MiraiBio, Bio-Rad or other Luminex instrument provider

Materials

Item	Source
Reagent Reservoirs, 25 mL and 100 mL capacities	Diversified Biotech (P/N RESE-3000, RESE-1000)
Procarta Transcription Factor Nuclear Extraction Kit or Whole Cell Lysis Kit	Panomics (P/N PC5101), (P/N PC5102)
Protein Determination Kit	Bio-Rad DC Protein Assay Kit (P/N 500-0112) or equivalent
Aluminum Foil	MLS

Guidelines for Assay Design and Data Analysis

Overview

Here we provide information and guidelines on the following:

- Preparing samples
- Optimizing sample input
- Running assay controls
- Running replicate samples
- Analyzing data

Note An example experimental plate layout is provided in "Appendix II" on page 22.

Preparing Samples

Protein concentration of sample inputs should be in the range of 400–2000 µg/mL. As a starting point, we recommend that each 10 µL sample contain a total of 2 µg of protein. For example, dilute samples to 0.2 µg/mL, using Working Sample Dilution Buffer, and then load 10 µL.

Optimizing Sample Input

We recommend running a 4-point, 2-fold serial dilution of the sample (diluting with Working Sample Dilution Buffer) to ensure you are operating in the linear range of the reader and assay.

For nuclear extracts, initially, we recommend preparing samples such that the addition of 10 µL of sample will contain a total of 2.0, 1.0, 0.5, and 0.25 µg of protein.

For whole cell lysates, initially, we recommend preparing samples such that the addition of 10 µL of sample will contain a total of 4.0, 2.0, 1.0, and 0.5 µg of protein.

Running Assay Controls

Assay Background Control

Assay background is the assay signal (median fluorescence intensity, MFI) generated by the assay components in the absence of sample.

We recommend you run an assay background control in every experiment and subtract the assay background MFI from each sample MFI when analyzing the data.

Whole Cell Lysate and Nuclear Extract Controls

Whole cell lysate and nuclear extract controls enable you to monitor assay performance. These controls are provided in the kit and are also available separately.

We recommend using at least one set of positive and negative controls in every experiment to monitor assay performance.

Running Replicates

Technical Replicates

These are replicates from a single extract or lysate sample.

Biological Replicates

These are replicates from different extracts or lysates but the extracts or lysates are biologically-equivalent.

Replicate Recommendations

For assays with 3–6 biological replicates, run 1 assay well/biological sample. For assays without biological replicates, run 3 technical replicate assay wells/biological sample.

Analyzing Data

Assay Precision

Run assays of the same sample in triplicate.

Calculate standard deviation and assay coefficient of variation (%CV = [std dev/mean] x 100%). Assay CVs are typical less than 15% for technical replicates.

Fold Change

Subtract average MFI background from all samples.

Calculate fold change as the treated sample value/untreated sample value.

Assay Controls

For the positive control, calculate the ratio of the NFκB signal (MFI) divided by the assay background. For NFκB, this value should be >20.

For each set of positive and negative controls, subtract the NFκB assay background from the NFκB signals in the controls. Then, divide the background-corrected NFκB signal from the positive control by the background-corrected NFκB signal from the negative control. This ratio, or fold change calculation should be >5.

Set-Up and Operation of the Vacuum Manifold System

About Using the Vacuum Manifold

This topic describes how to set up and use the Millipore vacuum manifold. This includes how to calibrate the pressure and important guidelines that will help to ensure good assay reproducibility.

We recommend that you set up and calibrate the manifold before you start the assay to ensure the assay is performed without interruption.

Sealing Filter Plates

- Lay a Plate Seal over the Filter Plate and roll a 5 mL serological pipet (or equivalent) over the Plate Seal to seal the Filter Plate.

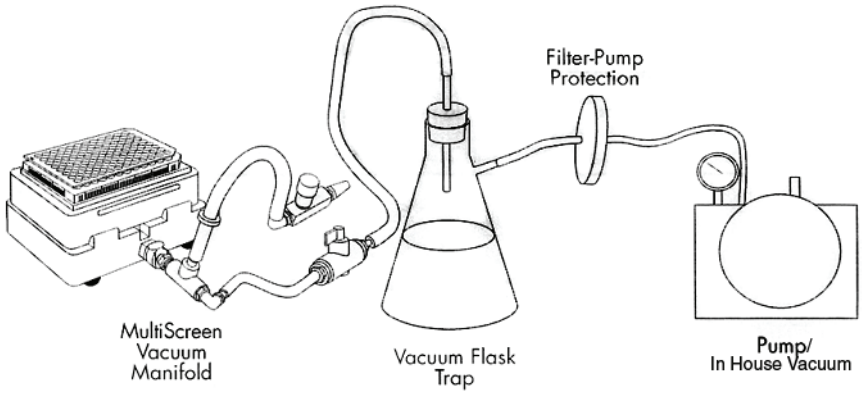
  This ensures adequate plate sealing while avoiding any leakage due to capillary action.

IMPORTANT To avoid Filter Plate leakages, do not seal Filter Plates using a rubber roller (or equivalent) as they apply significant pressure resulting in leakage.

- Seal all unused wells with the provided Plate Seal to ensure proper vacuum pressure.

Setting Up and Calibrating the Manifold

To set up and calibrate the manifold:

Step	Action
1	Set up the Filter Plate vacuum manifold as shown below. Follow the manufacturer's manual for details. 
2	Calibrate the vacuum pressure using the Utility Plate: a. Place the Utility Plate on top of the manifold. b. Turn on the vacuum. c. Press the corners of the Utility Plate to form a tight seal. d. Set the pressure to 2–3 mm of Hg. **IMPORTANT** During filtration, maintain the vacuum between 2–3 mm Hg. Higher vacuum may result in the loss of Capture Beads.

Operating the Manifold

To operate the manifold:

Step	Action
1	Once the vacuum is set correctly, remove the Utility Plate. Check vacuum calibration periodically. As a general guideline, 200 µL of solution should take approximately 2–5 seconds to clear the well of a Filter Plate.
2	For all filtration steps, turn the Filter Plate vacuum manifold on, transfer the Filter Plate to the vacuum manifold and then filter the solution. Avoid splashing and cross-contamination of wells during all wash steps. **IMPORTANT** During filtration, maintain the vacuum between 2–3 mm of Hg. Higher vacuum settings may result in loss of Capture Beads. **IMPORTANT** Do not allow the Filter Plates to air-dry following washes. Immediately add the next component following each filtration step.
3	Break the vacuum immediately after each solution has been completely filtered from all wells (approximately 2–5 seconds) by first turning off the vacuum, then removing the plate from the manifold. **Note** Wells typically filter at different rates.
4	Place the Filter Plate back on the Utility Plate.
5	Following the last wash in each series, blot the bottom of the Filter Plate thoroughly with a paper towel to remove traces of Wash Buffer. Avoid touching the bottom of the Filter Plate with your fingers or to the bench during manipulations.

Assay Procedure

Before You Start

- Prepare Working Sample Dilution Buffer. Based on your dilution calculations, prepare a Working Sample Dilution Buffer by combining 100 parts of Sample Dilution Buffer, 1 part DTT, and 1 part Protease Inhibitor Cocktail. Store on ice.
- Place Binding Buffer on ice.
- If Elution Buffer contains precipitates, warm to 37 °C with gently swirling.

Forming Protein-DNA Complexes

To form Protein-DNA complexes:

Step	Action
1	Thaw Detection Probes on ice.
2	If you are not using a whole plate, trim a PCR Plate Seal to the appropriate size and seal any unused wells of the PCR Plate.
3	Prepare the PCR Plate: a. Add 10 µL of Detection Probe to all experimental wells of the PCR Plate. b. Add 10 µL of Nuclear Extract Controls and/or Whole-Cell Lysate Controls to the appropriate assay wells. c. Add 10 µL of Working Sample Dilution Buffer to assay background control wells. d. Add 10 µL of prepared sample to each designated well. **Note** As a starting point, we recommend that each 10 µL sample contain a total of 2 µg of protein. Dilute samples with Working Sample Dilution Buffer.

To form Protein-DNA complexes: *(continued)*

Step	Action
4	Mix samples by gently tapping the bottom of the PCR Plate. **IMPORTANT** Make sure all bubbles are removed from the wells. (Tapping the plate should accomplish this.)
5	Incubate the plate at 15 °C for 30 minutes. **IMPORTANT** Make sure the temperature is maintained at 15 °C. We recommend using a PCR instrument. Samples can be kept at 4 °C for up to 1 hour before proceeding to the next step.

Eluting TF-Bound Detection Probes

To elute TF-bound detection probes:

Step	Action
1	Prepare Separation Plate: a. Trim a foil Plate Seal to the appropriate size and seal any unused wells of the Separation Plate. b. Place the Utility Plate on the bottom of the Separation Plate.
2	Pre-wet the Separation Plate: a. Add 180 µL/well ice-cold Binding Buffer to each well of the Separation Plate. b. Centrifuge the Separation Plate/Utility Plate assembly at 563 x g for 2 minutes at 4 °C. c. Discard the flow through and dry the top surface of the Utility Plate with a clean paper towel to avoid contaminating the bottom of the Separation Plate. **IMPORTANT** Do not place the Separation Plate directly on any surface other than the specified plates as this might result in cross contamination of wells. Place the Utility Plate on the bottom of the Separation Plate.
3	Transfer the samples to the Separation Plate: a. Add 20 µL ice-cold Binding Buffer to each well of the protein-DNA complexes in the PCR Plate. Avoid pipetting up and down. b. Transfer the entire 40 µL sample to the corresponding well of the Separation Plate. Make sure you apply the sample to the center of the filter.
4	With the Utility Plate on the bottom of the Separation Plate, incubate on ice for 30 minutes. **IMPORTANT** Do not exceed the incubation time as this will result in high background.
5	Wash the Separation Plate: a. Add 180 µL/well ice-cold Separation Buffer to each well of the Separation Plate. b. Incubate Separation Plate/Utility Plate assembly on ice for 5 minutes. c. Centrifuge the Separation Plate/Utility Plate assembly at 563 x g for 2 minutes at 4 °C. d. Discard the flow through and dry the top surface of the Utility Plate with a clean paper towel to avoid contaminating the bottom of the Separation Plate. e. Place the Separation Plate back on top of the Utility Plate.

To elute TF-bound detection probes: *(continued)*

Step	Action
6	Repeat step 5 (omitting step 5b) four more times for a total of 5 washes. **IMPORTANT** Do not reduce the number of washes as this will result in high background.
7	Centrifuge the Separation Plate/Utility Plate assembly at 563 x g for 3 minutes at 4 °C.
8	Elute TF-bound Detection Probes: a. Add 60 µL of Elution Buffer to the center of each experimental well in the Separation Plate. b. Seal unused wells of the Sample Collection Plate with a foil Plate Seal. c. Place the Separation Plate on top of the Sample Collection Plate and incubate at room temperature for 5 minutes. d. Centrifuge the Sample Collection/Separation Plate assembly at 563 x g for 3 minutes. You should have 60 µL/well in the Collection Plate. **IMPORTANT** Use a scale to balance the Sample Collection/Separation Plate assembly.
9	Place the samples on ice and continue to the next step, "Denaturing the Eluted Detection Probes" on page 15 or, cover with a foil Plate Seal and store at –20 °C until you are ready to use. Thaw on ice before use.

Denaturing the Eluted Detection Probes

IMPORTANT For 1-plate kits, there is sufficient reagents to process 1 entire plate using reagent reservoirs and multi-channel pipets. If you are running several partial plates, you may not have sufficient reagents for all steps. For 1/4-plate kits, there is not sufficient reagents to process the samples using reagent reservoirs and multi-channel pipets. For 1/4-plate kits, perform reagent additions using a single-channel pipet.

Before You Start

Turn on the Luminex-based reader at least 30 minutes before you intend to read your plate.

To denature the eluted Detection Probes:

Step	Action
1	Seal the samples wells in the Sample Collection Plate with a PCR Plate Seal. Using another PCR Plate Seal, cover any unused wells. **IMPORTANT** Do not use a foil Plate Seal as it will stick to the plate at high temperatures.
2	Denature samples at 95 °C for 5 minutes using a PCR instrument or heat block. Place samples on ice for 5 minutes.
3	Place the Filter Plate on top of the Utility Plate. Cut a foil Plate Seal to size and cover any unused wells on the Filter Plate.
4	Pre-wet the Filter Plate by dispensing 150 µL of Wash Buffer into each well and incubating the Filter Plate at room temperature for 5 minutes.
5	Remove the Wash Buffer using the vacuum manifold. When all Wash Buffer has been filtered, remove the Filter Plate from the vacuum manifold and blot the bottom of the Filter Plate with paper towels and place the Filter Plate on top of the Utility Plate.

To denature the eluted Detection Probes: *(continued)*

Step	Action
6	Proceed to the next step, “Hybridizing the Denatured Detection Probes” on page 16.

Hybridizing the Denatured Detection Probes

To hybridize the denatured Detection Probes:

Step	Action
1	Add premixed Capture Beads to the Filter Plate: a. Vortex the premixed Capture Bead solution at the highest setting for 30 seconds. b. Dispense 50 µL of premixed Capture Beads to each well of the FIlter Plate. c. Remove the Capture Bead buffer using the vacuum manifold. d. Blot the bottom of the Filter Plate with paper towels and place the Filter Plate on top of the Utility Plate. **IMPORTANT** Do not invert or tap the Filter Plate.
2	Wash the Capture Beads: a. Dispense 150 µL of Wash Buffer to each well of the Filter Plate. b. Remove the Wash Buffer using the vacuum manifold. c. Blot the bottom of the Filter Plate with paper towels and place the Filter Plate on top of the Utility Plate.
3	Dispense 40 µL of Assay Buffer to each well of the Filter Plate.
4	Add denatured Detection Probes (from “Eluting TF-Bound Detection Probes” on page 14): a. Transfer 20 µL of each eluted sample to each well of the Filter Plate according to your assay plate map. **IMPORTANT** Change the pipet tip after every transfer and avoid creating bubbles. b. Seal the wells of the Filter Plate with a foil Plate Seal. **IMPORTANT** Do not seal too tight as this may cause leaking. c. Place the Filter Plate on top of the Utility Plate.
5	Incubate the Filter Plate/Utility Plate assembly in a Vortemp shaking incubator set at 50 °C and 300–500 rpm for 30 minutes. **Note** If a shaking incubator is not available, wrap the Filter Plate/Utility Plate assembly with aluminum foil, shake the assembly at room temperature for 10 minutes, then transfer to a 50 °C incubator for 30 minutes without shaking.
6	Proceed to the next step, “Binding the SAPE” on page 17.

Binding the SAPE

To bind the SAPE:

Step	Action
1	Prepare to wash the Capture Beads: a. Remove the Plate Seal carefully to avoid splashing of samples. b. Remove the buffer using the vacuum manifold. c. Blot the bottom of the Filter Plate with paper towels and place the Filter Plate on top of the Utility Plate.
2	Wash the Capture Beads: a. Dispense 150 µL of Wash Buffer to each well of the Filter Plate. b. Remove the Wash Buffer using the vacuum manifold. c. Blot the bottom of the Filter Plate with paper towels and place the Filter Plate on top of the Utility Plate.
3	Add the Streptavidin-PE: a. Invert the Streptavidin-PE tube to mix. b. Dispense 100 µL of Streptavidin-PE to each well of the Filter Plate. c. Seal the Filter Plate with a foil Plate Seal. d. Wrap the Filter Plate/Utility Plate assembly with aluminum foil. e. Shake the assembly on a plate shaker at room temperature for 30 minutes at 300–500 rpm.

Detecting the Signal

To detect the signal:

Step	Action
1	Prepare to wash the Capture Beads: a. Remove the Plate Seal carefully to avoid splashing of samples. b. Remove the buffer using the vacuum manifold. c. Blot the bottom of the Filter Plate with paper towels and place the Filter Plate on top of the Utility Plate.
2	Wash the Capture Beads: a. Dispense 150 µL of Wash Buffer to each well of the Filter Plate. b. Remove the Wash Buffer using the vacuum manifold. c. Repeat step 2a-2b once more. d. Blot the bottom of the Filter Plate with paper towels and place the Filter Plate on top of the Utility Plate.
3	Resuspend Capture Beads: a. Dispense 120 µL of Reading Buffer to each well of the Filter Plate. b. Seal the wells with a Plate Seal. c. Wrap the Filter Plate/Utility Plate assembly with aluminum foil. d. Shake the Filter Plate at 300–500 rpm and room temperature for 5 minutes or until ready to read on the Luminex instrument. **Note** The Filter Plate/Utility Plate assembly can be wrapped with aluminum foil and stored flat, in the dark at 4 °C, for up to 48 hours before proceeding. However, delay in reading the plate may result in decreased sensitivity for some analytes. Shake the plate at 300–500 rpm for 5 minutes before reading.

To detect the signal: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>4</td><td>Read the plate:
a. Remove the Plate Seal carefully to avoid splashing of samples.
b. Read the plate using the following settings:

<table>
<tr><th>Sample Size</th><th>DD Gate</th><th>Timeout</th><th>Bead Events/Bead Region</th><th>Statistic</th></tr>
<tr><td>50 µL</td><td>8,000–15,000</td><td>25 seconds</td><td>50</td><td>Median</td></tr>
</table>

These settings are for the Luminex 100v 1.7 or the Luminex 100IS v2.1/2.2 Luminex or Bio-Plex instruments. If using other instruments, follow the manufacturer’s instructions.

IMPORTANT Check to ensure that the Luminex instrument probe height (needle) is adjusted appropriately for the Filter Plate.

IMPORTANT We recommend you calibrate the Luminex instrument each day you run the assay.

Note The Bio-Plex Suspension Array System allows calibration using Low or High sensitivity settings. Select the sensitivity during calibration, using pre-determined values of CAL2 RP1 target, as provided by Bio-Rad Laboratories. Using RP1 Low target value will provide results comparable to those obtained from the Luminex 100. Using the RP1 High target may increase detection sensitivity for low TF protein concentrations but sacrifice part of the linear dynamic range for high concentrations. We recommend RP1 High target value for Bio-Plex.</td></tr>
</table>

Troubleshooting

Possible Problems and Recommended Solutions

Observation	Possible Cause	Recommended Action
Filter plate leakage	Vacuum pressure too high	Adjust the vacuum pressure to 2–3 mm Hg as recommended in "Denaturing the Eluted Detection Probes" on page 15.
	Filter Plate is misaligned (at an angle) during incubation/processing	Set the Filter Plate/Utility Plate assembly on a flat, level surface during incubation/processing.
	Leakage from capillary action	After each vacuum step, blot the bottom of the Filter Plate using paper towels or absorbent paper.
	Plate Seal applied with too much force	Do not use a rubber roller to seal the plate.
High CV	Sample not prepared properly	Make sure the samples are eluted properly by using an accurate counterweight when centrifuging.
	Bottom of the Filter Plate is not dry	After each vacuum step, blot the bottom of the Filter Plate using paper towels or absorbent paper.
	Contamination from re-using the Plate Seal	Use a new Plate Seal for each incubation step.
	Contamination from Wash Buffer	Be careful not to splash Wash Buffer during wash steps into adjacent wells.
Low bead count	Volume of bead solution is incorrect	Make sure the volume of Capture Beads is correct.
	Beads are clumping	Vortex the bead solution well before using in the assay.
	Vacuum pressure too high	Use 2–3 mm Hg vacuum pressure.
	Filter ruptured due to excess vacuum time	Do not use the vacuum over 10 seconds in any of the steps.
	Dyes contained in the beads are photo-bleached from overexposure to light	Store bead solution and the assay plate in the dark
	Reader is clogged	Follow the instructions in the Luminex instrument user documentation.

Observation	Possible Cause	Recommended Action
Low signal or sensitivity	Low protein concentration	Increase the protein by at least 2-fold and retest.
	Samples were not kept on ice or Binding Buffer is not cold.	Keep samples and Binding Buffer on ice.
	Beads or reagents expired	Verify the expiration date of the kit.
	Beads stuck to the bottom of the plate	Make sure the plate is agitated at 300–500 rpm for the recommended time and for at least 5 minutes before reading.
High backgrounds	Incubation too long during elution of TF-bound Detection Probes	Do not exceed the 30 minute incubation time.
	Reduced number of wash steps during elution of TF-bound Detection Probes	Do not reduce the number of wash steps to less than 5.
Low or no fold induction observed	Protein concentration too low or too high	Optimize sample input.
	Signals from instrument are saturated	Reduce protein concentration of sample input.
	Target protein not activated (induced)	Review induction procedures. You may need to change cell lines, inducer, or induction conditions.

Contacting Panomics

Technical Help

For technical questions, contact our technical support group by telephone at 1-877-726-6642 option 3 or by email at techsupport@panomics.com (US and Canada) techsupport_europe@panomics.com (Europe), or visit our website www.panomics.com for an updated list of FAQs and product support literature.

For Additional Services

For information about Panomics products or for ordering information, contact your Regional Sales Manager, or visit our website at www.panomics.com.

Appendix I

Bead-Analyte Associations

The following tables provide the bead-analyte associations for setting your Luminex instrument. Refer to your product insert for analytes included in your kit.

Bead	Analyte	Bead	Analyte
7	Elk1	42	PPAR
11	NFκB	43	Smad
12	FAST1	44	RUNX/AML
17	Oct	45	Brn3
18	p53	46	CEBP
19	Pax3	47	NF-Y
20	NF-E2	51	c-myb
21	AP2	52	CREB
25	NF-E1(YY1)	53	ER
26	ATF2	54	GR/PR
27	NF-1	55	HIF-1
28	ISRE	56	FKHR
29	Pax5	61	GATA
32	Nkx-2.5	62	IRF
33	AR	63	Stat1
34	ETS/PEA	64	HNF1
35	AP1	65	STAT4
36	E2F1	66	STAT5
37	MyoD	73	MEF2
41	STAT3	76	NFAT

Appendix II

Sample and Blank Plate Layouts

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC[a] NE[b]		Sample A, 2 µg (from NE), treated			Sample B, 4 µg (from WCL), treated						
B	NC[c] NE		Sample A, 1 µg (from NE), treated			Sample B, 2 µg (from WCL), treated						
C	PC WCL[d]		Sample A, 0.5 µg (from NE), treated			Sample B, 1 µg (from WCL), treated						
D	NC WCL		Sample A, 2 µg (from NE), untreated			Sample B, 4 µg (from WCL), untreated						
E	Assay Back-ground		Sample A, 1 µg (from NE), untreated			Sample B, 2 µg (from WCL), untreated						
F			Sample A, 0.5 µg (from NE), untreated			Sample B, 1 µg (from WCL), untreated						
G												
H												

a. PC = Positive Control

b. NE = Nuclear Extract

c. NC = Negative Control

d. WCL = Whole Cell Lysate

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												