

KREX Protein Array

Image Scanning Settings For 1- and 4-plex Array Format

Instruction Manual



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1. Introduction

The KREX-based Protein Array is a slide based high-density protein microarray based on Sengenics patented KREX[™] protein folding technology (1). The product enables highly multiplexed detection and relative quantification of autoantibodies circulating in human blood and is intended primarily for disease biomarker discovery. The array content comprises ranges of antigens between 100+ and 1800+ immobilized, full-length, correctly folded human proteins. The proteins are immobilized on a proprietary, planar hydrogel surface supported by a glass slide. KREX[™] technology (1) ensures that only correctly folded proteins are immobilized onto the surface and the aqueous environment of the hydrogel helps the proteins to maintain their native conformation. The arrayed proteins represent major protein classes such as protein kinases and transcription factors, signalling molecules as well as proteins acting at the extracellular environment, such as cytokines.

Each array image from your KREX Protein Array experiment will be saved in a 16-bit TIFF format. Each spot on each array represents a protein or control probe immobilized on the array. Depending on the type of microarray scanner you are using, the amount of serum or plasma IgG bound to each protein on the array is typically represented in relative fluorescence units (RFU) or median fluorescence intensities (MFI). For the purpose of this manual the term 'relative fluorescence units (RFU)' will be used.

To obtain the RFU for each spot on the array, you will need to analyse each TIFF image using a compatible image analysis software*. This process involves converting each pixel within each spot on the array image into numerical values, i.e. RFUs. A GenePix Array List (GAL) file will be required to perform the image analysis. The GAL file contains the names and positions of all the proteins and control probes on each array. Our Support Team would have provided you with the relevant GAL file. You may also download the latest i-Ome[®] Protein Array GAL file from *https://sengenics.com/resources/product-information/*.

References

 Beeton-Kempen, N., Duarte, J., Shoko, A., Serufuri, J.-M., John, T., Cebon, J., & Blackburn, J. (2014). Development of a novel, quantitative protein microarray platform for the multiplexed serological analysis of autoantibodies to cancer-testis antigens. International Journal of Cancer, 135, 1842–1851



2. Settings Procedure

2.1 Quick Guide

Setting up slides scan region: Under the 'Tools' tab

- 1. Create scan region by choosing 'Tools > Scan Region Editor'.
- 2. Input the scanning dimensions and save.

Setting up slides scan protocol: Under the 'Tools' tab

- 1. Select an existing protocol as a template and click 'Save As'.
- 2. Write a new name and click 'Save'.
- 3. Choose scan settings for the protocol.
- 4. Click 'Save'.

Setting up Sengenics slides barcode and Agilent scan control program.

- 1. Click 'Open Door' and click 'Tools>Input Barcode'.
- 2. Scan the slide barcode, load the slide holder into the cassette and click 'Set'.
- 3. Next, click 'Close Door' after all slide holders have been loaded in the cassette.
- 4. Enter the slides barcode if the scanner unable to read it.
- 5. Choose 'Scan Protocol' and 'Output Folder'.
- 6. Click 'All to Queue' and 'Yes' to add the slides to the queue.
- 7. Click 'Start Scan' to begin scanning the slides.

Image Analysis

Data extraction from TIFF images using GenePix software.



2.2 Setting up scan region: Under the 'Tools' tab

The scan region determines the area of the slide that is scanned. Users can create their own scan region or change a scan region. The larger the region, the longer the scan time. The new region appears as a selection in the Protocol Editor.

1. Click 'Tools > Scan Region Editor' (Figure 1).

Sc	an Protocol Editor
Sc	an Region Editor
Inj	put Barcode
Sv	vitch on Lasers
Sh	now Recent Errors
Lo	g Files
Sta	ate Snapshot
Re	eset Calibration Warnings
Se	lf Test
Se	ttings

Figure 1: Scan Region Editor

- i. In the list next to 'Scan Region', choose 'FullStandardSlide' as a template.
- ii. Click 'Save As' and create a new name. Next click 'OK' (Figure 2).



Scan Region: FullSta	ndardSlide	-
⊿ General		
Locked	🔆 Save As New Name	×
✓ Scan Region		
TopLeft X	New Name: I-OMEV	0.0
	-	Consul OK
	-	Cancel OK
✓ Chip Package		
Package Name		StandardSlide
TopLeft X		2.6
TopLeft Y		1.9
		71
Height		21.6
TopLeft X Left edge position.	ave As Remove	Import Export Close



i. In 'Scan Region' section, input the measurements for the relevant array scanning region as depicted in Table 1 and Figure 3-6 below:

		Dimension (mn	n)	
Region	1-plex Array i-Ome Discovery (version 6.0)	4-plex Array CTA	4-plex Array OncoREX p53	4-plex Array Pan Autoimmune
Top left X	6.00	3.30	3.30	3.30
Top left Y	2.97	3.00	3.00	3.00
Width	67.00	70.00	70.00	70.00
Height	20.00	19.00	19.00	19.00

Table 1: Details of scan area for the following 4-plex array format

ii. Click 'Save' to save the changes for the selected scan region.



Scan Region: i-OMEv6.0_1	•
⊿ General	
Agilent Defined	
Locked	
Scan Region	
TopLeft X	6
TopLeft Y	2.97
Width	67
Height	20
Package Name	StandardClide
Topl aft Y	2.6
TopLeft Y	19
	71
	21.6

Figure 3: Setting up scan region for 1-plex array format, i-Ome® Discovery Protein Array



Figure 4: Dimension of scan region for 1-plex array



Scan Region:	CTA_2		
 ✓ General Agilent Defi Locked ✓ Scan Region 	ined		
TopLeft X TopLeft Y Width Height		3.3 3 70 19	
∠ Chip Packag	je		
TopLeft X TopLeft Y Width		2.6 1.9 71 21.6	

Figure 5: Setting up scan region for 4-plex array format







Figure 6: Dimension of scan region for 4-plex array format.



2.3 Setting slides scan protocol: Under the 'Tools' tab

A scan protocol is a predefined set of scan settings. Several default scan protocols are provided with the software. The 'Scan Protocol Editor' program allows users to select the fluorescence (dye) channels, scan regions, resolution, dynamic range, PMT gain and slides naming settings based on type of slides and assays.

1. Click 'Tools > Scan Protocol Editor' (Figure 7).

 is nep	
Scan Protocol Editor	
Scan Region Editor	
Input Barcode	
Switch on Lasers	
Show Recent Errors	
Log Files	
State Snapshot	- 1
Reset Calibration Warnings	
Self Test	
Settings	

Figure 7: Scan Protocol Editor

- i. Select any existing protocol as a template to create a new scan protocol.
- Click 'Save As' to save the existing protocol with a new name. Then 'Save As New Name' dialog box opens. Enter a new name for the protocol, and then click 'Save' (Figure 8).



				-
			V	
4	Scan Settings			
			Red+Green	
Þ			FullAgilentSlide	*
			5 um	
			16 bit	
			100	*
			100	Ŧ
		W. Cours As Ma		*
		INCH SAVE AS INF	w Name X	
4	Image Settings	Save As Ne	w Name X	
4	Image Settings Transform Image	New Name:	i-OMEv6.0 SC G 50	
4	Image Settings Transform Image Split	New Name:	i-OMEv6.0 SC G 50	
4	Image Settings Transform Image Split Compress	New Name:	i-OMEv6.0 SC G 50 Cancel OK	
4	Image Settings Transform Image Split Compress File Naming Settings	New Name:	i-OMEv6.0 SC G 50 Cancel OK	-
4	Image Settings Transform Image Split Compress File Naming Settings Field 1	New Name:	i-OMEv6.0 SC G 50 Cancel OK	
4	Image Settings Transform Image Split Compress File Naming Settings Field 1 Field 2	New Name:	i-OMEv6.0 SC G 50 Cancel OK	
	Image Settings Transform Image Split Compress File Naming Settings Field 1 Field 2 Field 3	New Nate:	i-OMEv6.0 SC G 50 Cancel OK Instrument SN Slide ID <none></none>	
	Image Settings Transform Image Split Compress File Naming Settings Field 1 Field 2 Field 3 Image File Info	New Nate:	i-OMEv6.0 SC G 50 Cancel OK Instrument SN Slide ID <none></none>	
	Image Settings Transform Image Split Compress File Naming Settings Field 1 Field 2 Field 2 Field 3 Image File Info File Name	New Nate:	i-OMEv6.0 SC G 50 Cancel OK Instrument SN Slide ID <none> <instrsn>_<slideid>_Sxxx.tif</slideid></instrsn></none>	
	Image Settings Transform Image Split Compress File Naming Settings Field 1 Field 2 Field 2 Field 3 Image File Info File Name Image Width (Pixels)	New Nate:	i-OMEv6.0 SC G 50 Cancel OK Slide ID Slide ID <none> <instrsn>_<slideid>_Sxx.tif 12200</slideid></instrsn></none>	
	Image Settings Transform Image Split Compress File Naming Settings Field 1 Field 2 Field 3 Image File Info File Name Image Width (Pixels) Image Height (Pixels)	New Nate	i-OMEv6.0 SC G 50 Cancel OK Instrument SN Slide ID <none> <instrsn>_<slideid>_Sxxtif 12200 4320</slideid></instrsn></none>	
	Image Settings Transform Image Split Compress File Naming Settings Field 1 Field 2 Field 3 Image File Info File Name Image Width (Pixels) Disk space required	New Nate:	i-OMEv6.0 SC G 50 Cancel OK Instrument SN Slide ID <none> <instrsn>_<slideid>_Sxoc.tif 12200 4320 201.05 MB</slideid></instrsn></none>	

Figure 8 Create a new scan protocol.



iii. Change the scan, image, and file naming settings according to the slides and assay types. For example, if user run i-Ome[®] slides and Single Colour assay, the scanning protocol settings will be set as shown in Figure 9 below.

▲ General		
Agilent Defined		
Locked		_
Dve Channel(s)	Green	•
Scan Begion	i-OMEv6.0	•
Resolution	10 um	•
Tiff Dynamic Range	16 bit	•
Red PMT Sensitivity (%)	50	•
Green PMT Sensitivity (%)	50	•
XDR Ratio	<noxdr></noxdr>	•
Image Settings		
Transform Image	Flip/Rotate	•
	No	
Compress	No	•
 File Naming Settings 		
Field 1	Instrument SN	•
Field 2	Slide ID	•
D Field 3	Slot Number	•
A Image File Info		
	<instrsn>_<slideid>_<slotnum>_Sxxx.tif</slotnum></slideid></instrsn>	
	6700	
	2000	
Disk space required	25.56 MB	
	11 min	

Figure 9 Scan protocol settings (Image example for 1-plex array format, single colour)



NB: For standard scans, the Scan Control program uses up to three user-defined name prefixes to compose the file name. These prefixes are defined in the scan protocol. Scan files are named using the following rules: 'Field1_Field2_Field3_ScanNumber.tif'.

For example, US4510PP02_251485023883_S001_S03.tif

Field 1: Instrument Serial # = US4510PP02

Field 2: Slide ID = 251485023883

Field 3: Slot Number = S001

S03 is a Scan Number which indicates the third scan file in the folder with the same Instrument Serial # and Slide ID.

iv. Click 'Save' when the settings are completed. See example in figure 10-11 below:

-	Scan Protocol: i-OMEv6.0 SC G 50	•	
4	General		
	Locked		
4	Scan Settings		
	Dye Channel(s)	Green	•
Þ	Scan Region	i-OMEv6.0	•
	Resolution	10 um	•
	Tiff Dynamic Range	16 bit	•
	Red PMT Sensitivity (%)	50	•
	Green PMT Sensitivity (%)	50	•
	XDR Ratio	<noxdr></noxdr>	•
4	Image Settings		
	Transform Image	Flip/Rotate	٠
		No	
	Compress	No	٠
4	File Naming Settings		
Þ	Field 1	Instrument SN	•
Þ	Field 2	Slide ID	•
Þ	Field 3	Slot Number	•
4	Image File Info		
	File Name	<instrsn>_<slideid>_<slotnum>_Sxxx.tif</slotnum></slideid></instrsn>	
		6700	
		2000	
	Disk space required	25.56 MB	
		11 min	

Figure 10: Saved scan protocol (image example for 1-plex array, i-Ome Discovery Array)



₄ General	
Agilent Defined	
Locked	
 Scan Settings 	
Dye Channel(s)	Green
Scan Region	CTA_2
Resolution	10 um
Tiff Dynamic Range	16 bit
Red PMT Sensitivity (%)	50
Green PMT Sensitivity (%)	50
XDR Ratio	<noxdr></noxdr>
Image Settings	
Transform Image	Flip/Rotate
	No
Compress	No
File Naming Settings	
Field 1	Instrument SN
Field 2	Slide ID
Field 3	Slot Number
Image File Info	
File Name	<instrsn>_<slideid>_<slotnum>_Sxxx.tif</slotnum></slideid></instrsn>
	7000
	1900
Disk space required	25.37 MB
Scan time	11 min

Figure 11: Saved scan protocol (image example for 4-plex array, CTA Array)



2.4 Setting up Sengenics slides barcode and Agilent scan control program.

1. In the Scan Control program window, click 'Open Door' to open the scanner door (Figure 12).

Help			
Slide 1D	State Scan Protocol	Output Folder	
			Empty Queue
			One Deer
			openoour
			Start Scan
tus Log Scar	log		
:02:48	Calibrating ADC.		
:02:51	Calibrating 'Red' channel PMT. Calibrating 'Green' channel PMT.		
102153	Ejecting slide.		
104146	Warming up lasers.		

Figure 12: Agilent Scan Control window

2. Insert a slide into a slide holder.



3. Click 'Tools > Input Barcode' (Figure 13).

Note: If you are using external barcode reader, please ensure the barcode reader is connected to the computer. Please ensure to set or change scan data format to 'Data As Is' or 'Return to Factory Defaults'. Kindly please refer to the barcode reader manufacturer's manual.



Figure 13: Input Barcode



4. A pop-up message will appear. Scan the barcode, load the slide holder into the cassette and click 'Set'. The barcode is displayed under Slide ID in the Scan Control software slot table (Figure 14-15).

	Slide ID	State	Scan Protocol	Output Folder
				🖗 Input Barrode
				* information
				Enter a barcode, load the slide into slot 1, then click 'Set'.
				Barcode: 016077 Set
_				
	_			Close
in la				Close
tus Lo Tools	G Scan Lo	a		Close
tus Lo Tools	9 Scan Lo Help	0		Close
tus Lo Tools	G Scan Lor Help Slide ID	o State	Scan Protocol	Close
tus Lo Tools	Side ID 016077	9 State Present	Scan Protocol	Close DiScan Data DiScan Data
tus Lo Tools	 Scan Los Help Slide ID 016077 016078 014983 	9 State Present Present	Scan Protocol	Close DiScan Data DiScan Data DiScan Data
tus Lo Tools	9 Scan Lo Help 016077 016078 014983 014990	9 State Present Present Present Present	Scan Protocol	Close DiScan Data DiScan Data DiScan Data
tus Lo Tools	 Scan Lor Help Slide ID 016077 016078 014983 014990 	9 State Present Present Present Present	Scan Protocol	Output Folder DiScan Data DiScan Data DiScan Data DiScan Data
tus Lo Tools	Scan Lor Help Slide ID 016077 016078 014983 014990	D State Present Present Present Present	Scan Protocol	Close DiScan Data DiScan Data DiScan Data DiScan Data DiScan Data
tus Lo Tools	Scap lot Help Slide ID 016077 016078 014983 014990	o State Present Present Present Present	Scan Protocol	Close DiScan Data DiScan Data DiScan Data DiScan Data
tus Lo Tools	Scan Los Help Slide ID 016077 016078 014983 014990	Present Present Present Present Present	Scan Protocol	Output Folder DiScan Data DiScan Data DiScan Data DiScan Data
tus Lo Tools	 Scan Lee Help Slide ID 016077 016078 014983 014990 	n State Present Present Present Present	Scan Protocol	Close DiScan Data DiScan Data DiScan Data DiScan Data
tus Lo Tools	 Scan Let Help Slide ID 016077 016078 014983 014990 	n State Present Present Present Present	Scan Protocol	Output Folder DiScan Data DiScan Data DiScan Data
tus Lo Tools	 Scan Let Help Slide (D) 016077 016078 014983 014990 	O State Present Present Present Present	Scan Protocol	Output Folder DiScan Data DiScan Data DiScan Data DiScan Data
tus Lo Tools 117 117 117 117 117 117 117 117 117 11	 Scan Lof Help Slide ID 016077 016078 014983 014990 	State Present Present Present Present	Scan Protocol	Close DiSan Data DiSan Data DiSan Data DiSan Data
tus Lo Tools	 Scan Lo Help Slide ID 016077 016078 014993 014990 	D State Present Present Present Present	Scan Protocol	Output Folder DiScan Data DiScan Data DiScan Data
tus Lo Tools 117 127 127 127 127 127 127 127 127 127	 Scan Lot Help Slide ID 016077 016078 014990 014990 	State Present Present Present Present	Scan Protocol	Output Folder DiScan Data DiScan Data DiScan Data DiScan Data
	 Scon Let Help Stide ID 016077 016078 014983 014990 	State Present Present Present Present	Scan Protocol	Close
tus Lo Tools 117 117 117 117 117 117 117 117 117 11	 Scan Lo Help Stide ID 016077 016078 014983 014990 	C State Present Present Present Present	Scan Protocol	Close DiScan Data
tus Lo Tools 117 127 127 127 127 127 127 127 127 127	Sran Lee Help Slide ID 016077 016078 014983 014990	State Present Present Present Present Present	Scan Protocol	Close DiScan Data DiScan Data DiScan Data DiScan Data DiScan Data DiScan Data
tus Lo Tools 117 127 127 127 127 127 127 127 127 127	 Sran Let Help Slide ID 016077 016078 014983 014990 	State Present Present Present Present	Scan Protocol	Close
tus Lo Tools 10 10 10 10 10 10 10 10 10 10 10 10 10	Scon Lot Help Side ID 016072 016078 014983 014990	State Present Present Present Present	Scan Protocol	Close DiScan Data
tus Los Tools 117 117 117 117 117 117 117 117 117 11	C Scan Lot Help 016077 016078 014983 014990	State Present Present Present Present Present	Scan Protocol	Close DiScan Data
Lus Los Tools 10 10 10 10 10 10 10 10 10 10 10 10 10	 Scenario Help Side ID 016077 016078 014993 014990 	State Present Present Present Present	Scan Protocol	Close DiScan Data
tus Lo Tools	Scanicz Help Slide ID 016077 016078 014990	State Present Present Present Present	Scan Protocol	Close DiScan Data
tus Lo Tools 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	C Scan Lot Help 016077 016078 014990	State Present Present Present Present	Scan Protocol	Close DiScan Data

Figure 14 Scan the slides barcode before start scanning.





Figure 15: Place the slide holder into the cassette.

- 5. Repeat step 2 and 4 until all slides are loaded in the cassette.
- 6. Next, click 'Close Door'.
- 7. If the scanner could not read the barcode on slides, enter the barcode manually by clicking the Slide ID cell for the specific slide.
- 8. For each slide in the slot table, click the 'Scan Protocol' and select a scan protocol to use for the scanning processes.
- 9. Next, choose the output folder. The output folder is a location of the scanned image files for slides are saved. By default, the output folder is directed to D:\ScanData.
- 10. Click the 'Output Folder' cell for a slide and click the browse icon.
- 11. Browse to the location where you plan to save the scanned image files and click 'OK'.
- 12. If all the scanning settings have been set up and the 'State' column showed 'Ready for queue', click 'All to Queue' and click 'Yes' to add the slides to the scanning queue.

Note: To add a slide to the scan queue, its 'State' column must be select to 'Ready to Queue' (this status is typically autodetected)



- 13. Click 'Start Scan' to begin scanning the slides.
- 14. If the scanner status showed 'LaserOff', user need to warm up the laser first before proceeding with scanning (Figure 16).
- 15. Click 'Tools > Switch on laser' (Figure 17).

4	Scan Settings	
	Dye Channel(s)	Ψ
₽	Scan Region	· · · · · · · · · · · · · · · · · · ·
	Resolution	~
	Tiff Dynamic Range	
	Red PMT Sensitivity (%)	· · · · · · · · · · · · · · · · · · ·
	Green PMT Sensitivity (%)	-
	XDR Ratio	~
	Image Settings	
	Transform Image	Ŧ
	Split	*
	Compress	· ·
4	File Naming Settings	
₽	Field 1	-
₽	Field 2	
₽	Field 3	Ψ
So	can Description	_
U	ser	
Disl	c space required: 0 KB	LasersOff

Figure 16: Lasers Off Status



🔆 Agilent Microarray Scan Control	
Tools Help	
Scan Protocol Editor	Scan Protocol
Scan Region Editor	Scall Flottocol
Input Barcode	
Switch on Lasers	
Show Recent Errors	
Log Files	
State Snapshot	
Reset Calibration Warnings	
Self Test	-
Settings	
14/	
16/	
17 /	
18/	
19/	
20/	
[22]	
23/	
24/	

Figure 17: Switch on lasers

16. Proceed with Image Analysis after scanning process has completed.

* We do not provide any microarray image analysis software with any of our products. We highly recommend using GenePix® Pro 7 Software (https://support.moleculardevices.com).



2.5 Troubleshooting

1. To flip an image in Agilent Feature Extraction software, open an image, go to 'Tools' in the menu bar and select the preferred direction of flip. Use the orientation marker as guideline:





2. To adjust the color contrast of an image in Agilent Feature Extraction software, select the Auto Color Scaling drop down menu and select the preferred laser channel to adjust the color contrast. Fill in various ascending or descending number under the 'Maximum' box at either red or green color scale until signal is observed. This will not change the image original image as this meant for you to inspect the slide post scan. Further color adjustment can be done in GenePix.



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