Advanta IO Gene Expression Assay

Frequently Asked Questions

What is the Advanta IO Gene Expression Assay?

The Advanta™ IO Gene Expression Assay qPCR assay panel enables profiling of tumor immunobiology and identification of new biomarkers. It was designed to meet the rigorous demands of human checkpoint research programs. It includes key markers of tumor immune response that were shown in a multicenter international clinical trial to inform tumor progression and checkpoint therapeutic response. See Herbst, R.S. et al., *Nature* 515 (2014): 563–7 and Fehrenbacher, L. et al., *The Lancet* 387 (2016): 1,837–46.

In collaboration with leading researchers and biopharmaceutical companies, Fluidigm further expanded this panel to include 74 additional immuno-oncology markers.

How many genes are in the panel?

170 genes are assigned to two sets, designated as Panel A and Panel B. Panel A contains 91 genes and 5 housekeeping genes. Panel B contains 74 genes and the same 5 housekeeping genes.

What are the genes in the panel?

The complete panel set includes genes for identification and functional analysis of immune and cancer cells, including markers found in defined T cell subsets, cytokines, and chemokines and markers of immune regulation, immune cell fate, and more. All genes are identified by their official gene symbols. Some genes are better-known by their aliases, which appear in parentheses.

Panel A:

ARG1	CLEC4C	IL12A	PDCD1LG2 (PD-L2)
BTLA	CSF2	IL13	PRF1
CCL2	CTLA4	IL17A	PTGER2
CCL22	CX3CL1	IL17F	PTGER4
CCL28	CXCL10	IL1B	PTGS2
CCR5	CXCL8	IL2	PTPRC
CCR7	CXCL9	IL2RA	RORC
CD1C	CXCR3	IL4	SDHA
CD244	EOMES	IL6	SP2
CD27	EPCAM	IL7	TBX21
CD274 (PD-L1)	FOXP3	IL7R	TGFB1
CD276	GZMA	ITGAM	TMEM55B
CD28	GZMB	ITGAX	TNF
CD3E	HAVCR2	ITGB2	TNFRSF14
CD4	HLA-A	KLRK1	TNFRSF4
CD40	HLA-B	LAG3	TNFRSF9
CD40LG	HLA-C	LGALS9	TNFSF4
CD48	HMOX1	MAP4K1	TNFSF9
CD69	ICAM1	MICA	VCAM1
CD70	ICOS	MICB	VEGFA
CD80	IDO1	MS4A1	VPS33B
CD86	IFNG	NCAM1	VTCN1
CD8A	IL10	PDCD1 (PD-1)	

Panel B:

APOBEC3A	CXCR4	IFNA2	NKG7
APOBEC3B	CYBB	IGHA1	NRAS
ARG2	DGAT2	IGHG1	NT5E
CA4	EBI3	IGHM	PYGL
CCL18	ERBB2	IGKC	SLAMF7
CCL21	FASLG	IGLJ3	SLAMF8
CCL3	FCER1G	IGSF6	STAT1
CCL4	FCRLA	IL10RA	STAT2
CCL5	FYB	IL12B	STAT3
CD160	GATA3	IL15	STAT5A
CD19	GNLY	IL2RG	STAT5B
CD1D	GZMH	IRF9	STAT6
CD2	GZMK	ISG15	TLR7
CD22	HLA-DMB	JAK2	TLR8
CD37	HLA-DPB1	JCHAIN (IGJ)	TNFAIP8
CD52	HLA-DQB1	KREMEN1	TNFRSF18
CD53	HLA-DRB1	LAPTM5	TNFSF18
CD63	IFI27	LCK	
CTSS	IFIT2	LRG1	

Reference genes (within Panel A and Panel B):

B2M, ACTB, GAPDH, GUSB, TFRC

What chemistry is used for the panel?

Thermo Fisher Scientific Applied Biosystems® TaqMan® probes and primers.

Is it possible to add assays to the panel?

Yes, 17 open assay inlets on Panel B are available for additional TaqMan assays. Assays (at 20X concentration) can be sourced directly from Thermo Fisher Scientific.

Do I have to purchase both of the subpanels in the panel set?

No, we offer configurations of the Advanta IO Gene Expression Assay that support either panel separately or both panels as a set.

Is preamplification required?

Yes, preamplification is required to achieve the performance characteristics of the assay. The protocol includes instructions for performing preamplification, and each kit includes the required reagents and pooled primers. The protocol also provides instructions for adding up to 17 custom TaqMan Assays (each at 20X concentration) to the preamplification pool for Panel B.

What is the Advanta IO Gene Expression Control 1 and how is it used?

The Advanta IO Gene Expression Control 1 (PN 101-7676) is a synthetic control designed to qualitatively ensure that all assays are working. All assays are expected to generate signal within the target Cq range of 8–22 when the control is used according to protocol. The control is not intended to gauge assay performance or quality information from natural, biologically derived samples. The control is sold separately, and use of it is optional.

I already have IFCs. Can I just buy the assays?

No, the reagents included in the Advanta IO Gene Expression Assay have been analytically validated together to ensure optimal performance across all genes. These reagents are different than the off-the-shelf gene expression reagents available from Fluidigm. Each kit contains all of the reagents and IFCs (integrated fluidic circuits) necessary to test the panel.

Which IFC is used for the panel?

We offer configurations of the Advanta IO Gene Expression Assay that support the 96.96 and 24.192 Dynamic Array™ IFCs for Gene Expression (GE 96.96 and GE 24.192 IFCs).

What equipment is required to use the product?

The Biomark™ HD system and the IFC Controller HX (for 96.96) or RX (for 24.192) or Juno™ system (for both 96.96 and 24.192).

What reagents are required to run the panel?

The Advanta IO Gene Expression Assay is available in several configurations, where all required reagents are included:

- Reverse Transcription Master Mix
- Preamp Master Mix
- 20X GE Sample Loading Reagent
- 2X Assay Loading Reagent
- Gene Expression Master Mix
- PCR Water
- Dilution Reagent
- Rehydration Solution
- Advanta IO GE Assay Plate (Panel A, Panel B, or both panels)
- Advanta IO GE Preamp Pool (Panel A, Panel B, or both panels)
- Actuation and Pressure Fluids (for kits that use the 24.192 Dynamic Array IFC)

How many samples can be processed in a single run?

The number of samples depends on the IFC format and the number of replicates per sample, as outlined below.

Replicates per Sample	Samples per GE 24.192 IFC	Samples per GE 96.96 IFC
1	24	96
2	12	48
3	8	32
4	6	24

How many assays can be included in a single run?

The number of assays depends on the IFC format. All samples run on a single GE 96.96 IFC are tested by up to 96 assays, and all samples run on a single GE 24.192 IFC are tested by up to 192 assays. As applied to the Advanta IO Gene Expression Assay, running Panels A and B requires the use of either two GE 96.96 IFCs or one GE 24.192 IFC.

How many runs can be accomplished in a single day?

Two to three runs, depending on operational setup.

What is the assay workflow?

Assay workflow for the GE 96.96 IFC

	Workflow Step		Hands-On Time	Run Time	Total Time
1	**Prepare the cDNA.** Reverse transcription reaction	Potential stopping point	15 min	40 min	**55 min**
	While RT is running: a (Optional) Prepare preamplification pools for user-defined assays. b Prepare the preamplification reaction.		15 min		
2	**Preamplify the cDNA.**	Potential stopping point	—	1 h 20 min*	**1 h 20 min**
	While preamplification is running: a Prime the GE 96.96 IFC.		5 min	20 min	
	b Rehydrate dried-down assays. c (Optional) Prepare user-defined assays.		20 min	—	
3	**Prepare sample mixes.**		20 min	—	**20 min**
4	**Load the GE 96.96 IFC.** Juno™ or IFC Controller HX.		5 min	1 h 30 min	**1 h 35 min**
5	**Thermal-cycle and collect data** Biomark HD		5 min	2 h 15 min	**2 h 20 min**
	Total		**1 h 25 min**	**5 h 45 min**	**6 h 30 min**

* For 14 cycles

Workflow for the GE 24.192 IFC

	Workflow Step		Hands-On Time	Run Time	Total Time
1	**Prepare the cDNA.** Reverse transcription reaction	Potential stopping point	15 min	40 min	**55 min**
	While RT is running: a (Optional) Prepare preamplification pools for user-defined assays. b Prepare the preamplification reaction.		15 min		
2	**Preamplify the cDNA.**	Potential stopping point	—	1 h 20 min*	**1 h 20 min**
	While preamplification is running: a Rehydrate dried-down assays. b (Optional) Prepare user-defined assays.		20 min	—	
	c Prepare assays and sample dilution buffer.		20 min	—	
3	**Dilute the preamplified cDNA and prepare final sample mixes.**		5 min	—	**5 min**
4	**Load the IFC.** Prime, load, and mix (one step) on Juno™ or IFC Controller RX.		5 min	30 min	**35 min**
5	**Thermal-cycle and collect data** Biomark HD		—	1 h 30 min	**1 h 35 min**
	Total		**1h 20 min**	**4 h**	**4 h 30 min**

* For 16 cycles

What level of skill is required to perform the assay (workflow complexity)?

General molecular biology lab techniques, including the use of multichannel pipettes.

What level of manual input is required?

Approximately 1.3 hours of manual hands-on time is necessary for the assay. Manual steps are of minimal complexity and are bracketed by periods of unattended processing/automation. Refer to the assay workflow overview for more details.

Can I automate use of the product with my liquid handler?

Yes. Contact techsupport@fluidigm.com to schedule a meeting with your field application scientist for assistance.

What additional materials are needed to use the kit?

All necessary reagents, assays, and IFCs are included in the kit.

Which software version should I use?

Use Biomark Data Collection software (version 4.5.1 or up), which supports use of the GE 24.192 IFC and enables you to select multiple assays (detectors) as reference for differential expression calculations. See the Real-Time PCR Analysis User Guide (PN 68000088) for more information.

Which genes should I use as reference to normalize expression?

Five reference genes included in both Panel A and Panel B may be used as reference genes. The expression level of genes selected as a reference should be independent of the conditions under investigation.

How should I perform data analysis?

Use the Real Time PCR Analysis software (version 4.5.1 or up) to conduct primary data analysis.

What analysis settings should be used?

Use **Linear (Derivative)** for baseline correction and **User (Detectors)** with Initialize with Auto for Ct Threshold Method. See the Real-Time PCR Analysis User Guide (PN 68000088) for more information.

What are the performance characteristics?

Sensitivity: single copy

RNA specificity: >97%

Dynamic range: 6 logs

What is the sample RNA input amount?

2–200 ng of total RNA.

What sample types are supported?

Purified RNA from FFPE, fresh or fresh frozen tissue.

Is this a single-cell assay?

No, it is for tissue.

What are the part numbers for ordering?

Content: Reagents, assays, preamplification pools, syringes, IFCs

Product Part Number	Product Description
101-6082	Advanta IO Gene Expression Assay—Panel A, GE 96.96, 2 IFCs
101-6083	Advanta IO Gene Expression Assay—Panel B, GE 96.96, 2 IFCs
101-6084	Advanta IO Gene Expression Assay—Panels A & B, GE 96.96, 4 IFCs
101-7678	Advanta IO Gene Expression Assay—Panels A & B, GE 24.192, 2 IFCs

How do I contact Support?

Visit fluidigm.com/support or email support@fluidigm.com.

For technical support visit fluidigm.com/support.

North America +1 650 266 6100 | Toll-free (US/CAN): 866 358 4354 | techsupport@fluidigm.com **Latin America** +1 650 266 6100 | techsupportlatam@fluidigm.com

Europe/Middle East/Africa/Russia +44 1223 598100 | techsupporteurope@fluidigm.com **China (excluding Hong Kong)** +86 21 3255 8368 | techsupportchina@fluidigm.com

Japan +81 3 3662 2150 | techsupportjapan@fluidigm.com **All other Asian countries/India/Australia** +1 650 266 6100 | techsupportasia@fluidigm.com