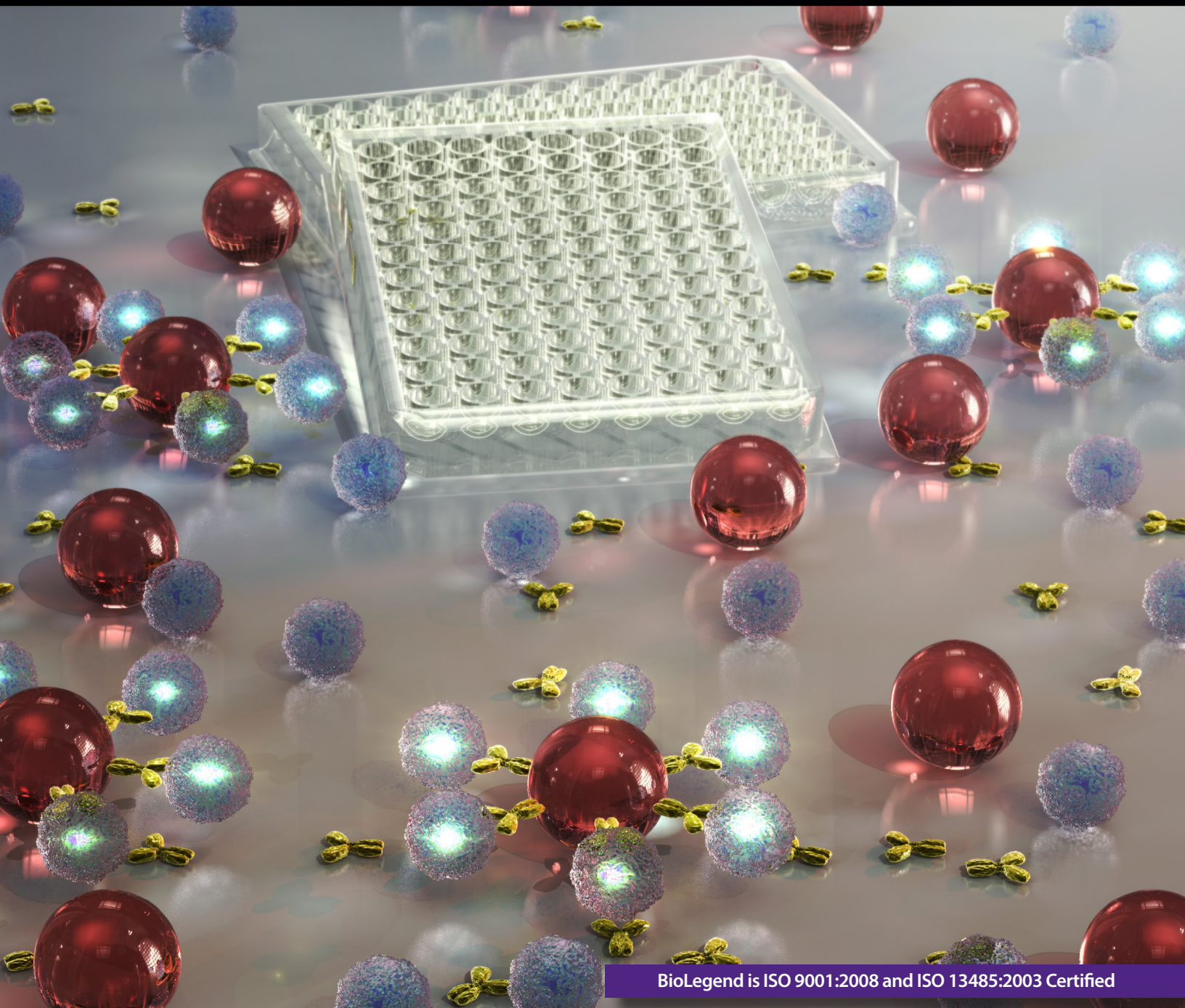


LEGENDplex™

Multi-Analyte Flow Assay Kits



BioLegend is ISO 9001:2008 and ISO 13485:2003 Certified



Toll-Free Tel: (US & Canada): 1.877.BIOLEGEND (246.5343)

Tel: 858.768.5800

biolegend.com

02-0009-02

World-Class Quality | Superior Customer Support | Outstanding Value

LEGENDplex™ Multi-Analyte Flow Assay Kits

LEGENDplex™ Multi-Analyte Flow Assay Kits are bead-based immunoassays for simultaneous quantification of multiple analytes in a single sample using a standard flow cytometer. Free, intuitive software enables fast and easy analysis of your data.

Kits can be ordered as either Pre-defined Panels (*e.g.*, Th1, Th2, Th17) or Mix and Match (choose your own specificities within a panel). The reagents are provided in a ready-to-use format, saving reagent preparation time.

LEGENDplex™ features:

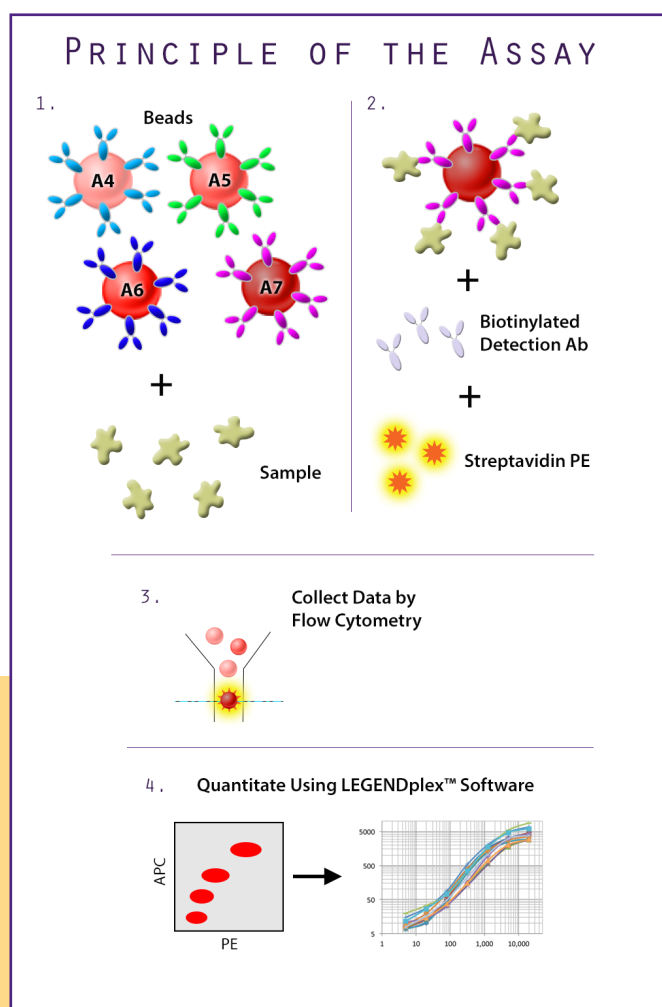
- Multiplex bead assays on common flow cytometers
- Pre-defined Panels or Mix and Match System
- Free data analysis software compatible with both Mac and Windows
- Affordability
- Flexibility; compatible with V-bottom plates (low-binding), and filter plates.

Items included in the kit:

- Setup beads
- Capture beads
- Matrix, lyophilized
- Detection antibody mix
- Standard cocktail, lyophilized
- All of the required buffers
- Filter plate or V-bottom plate and plate sealers
- Data analysis dongle
- Manual

Required items not included in the kit:

- Flow cytometer
- Pipettes and tips
- Reagent reservoirs for multichannel pipettes
- Polypropylene microfuge tubes
- Vortex mixer
- Sonicator (Optional)
- Plate shaker
- A vacuum filtration unit and a vacuum source (if using filter plates)
- Centrifuge



BioLegend will continue to expand the selection for LEGENDplex™ products. To stay updated on these releases, visit: [biolegend.com/legendplex](https://www.biolegend.com/legendplex)

Pre-defined Panels

The pre-defined panels provide all the reagents you'll need to run LEGENDplex™, including the setup beads, capture beads, matrix, detection antibody mix, standard cocktail, buffers, filter plates or V-bottom plates, and plate sealers.

Mix and Match

Use the LEGENDplex™ Mix and Match System if you are interested in ordering a subset of specificities within a panel. In order to use this system, you will need to order capture beads, the appropriate buffer set, standard, and detection antibodies. Depending on the panel, you may also need to order a V-bottom or a filter plate if the buffer set does not include one. This system is easy to use and cost-effective.

It is important to note that Mix and Match can only be done within panels. If you would like to combine specificities across different panels, you can make a request to our Custom Solutions Team at: cst@biolegend.com.

How to Mix and Match

Choose Capture Bead Specificities: Be sure to choose specificities within the panels for each assay. If you would like to mix specificities from different panels into a single assay, our Custom Solutions Team will be able to help, otherwise they should be split into multiple assays. Verify that a Pre-defined Panel does not already exist, as these will be most cost-effective.

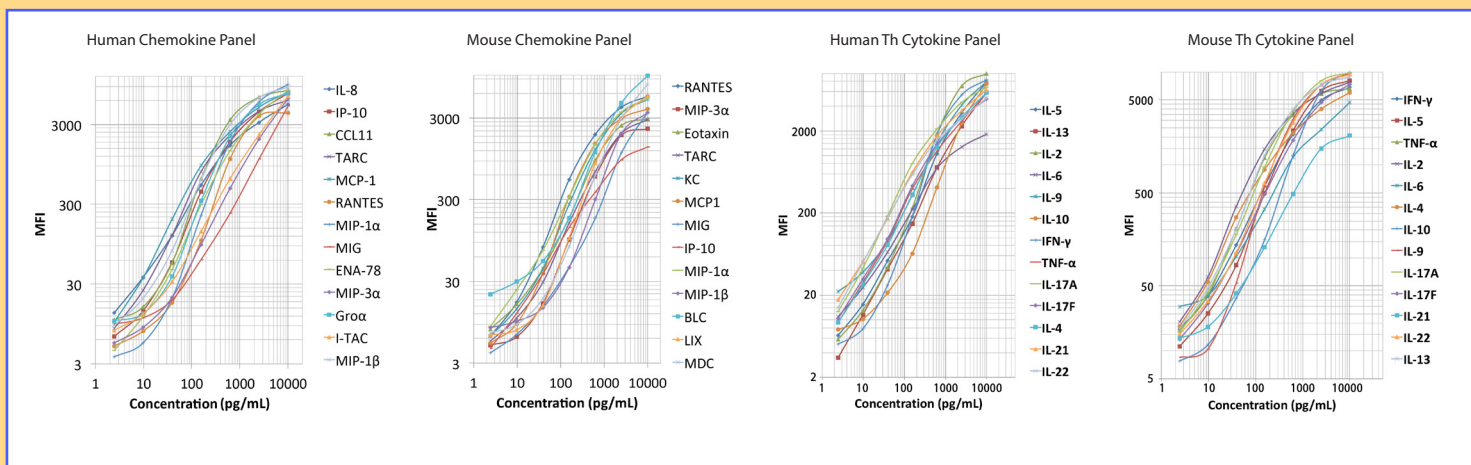
Add Standard (lyophilized) to your order: Each panel requires a single lyophilized standard mixture that contains standards for all specificities in the panel. Only one standard is required per assay.

Add Detection Antibodies to your order: Each panel requires a single mixture of Detection Antibodies. Only one Detection Antibodies product is required per assay.

Add a Buffer Set to your order: Each panel requires a Buffer Set.

Add a Plate to your order (if necessary): Some panels' Buffer Set includes a Plate, while other panels require a Plate to be added, as noted in product tables. Refer to the FAQ section of this brochure to decide which plates are appropriate for your use.

Example Data



Human Panels

Human T Helper Cytokine Panels *(Includes Filter Plate; V-bottom plate version coming soon)*

Pre-defined Panels include ALL required components and ALL check-marked targets										Mix & Match Capture Beads Cat. No.
Cat. No.		740001	740009	740011	740013	740015	740019	740021	740023	
Target	Bead ID	Th	Th1	Th2	Th1/Th2	Th17	Th9	Th22	Tfh	
IFN- γ	B2	✓	✓		✓	✓				740052
IL-2	A6	✓	✓		✓					740041
IL-4	B6	✓		✓	✓					740042
IL-5	A4	✓		✓	✓					740043
IL-6	A7	✓	✓	✓	✓	✓		✓	✓	740044
IL-9	A8	✓					✓			740045
IL-10	A10	✓	✓	✓	✓	✓	✓	✓	✓	740046
IL-13	A5	✓		✓	✓					740047
IL-17A	B4	✓				✓				740048
IL-17F	B5	✓				✓				740049
IL-21	B7	✓				✓			✓	740050
IL-22	B9	✓				✓		✓		740051
TNF- α	B3	✓	✓	✓	✓	✓	✓	✓	✓	740053
Required Mix and Match Components (already included in Pre-defined Panels)										
Standard										Cat. No. 740397
Detection Antibodies										Cat. No. 740071
Buffer Set (includes Filter Plate)										Cat. No. 740075

Human Cytokine Panel 2 *(Includes Filter Plate)*

Pre-defined Panel Cat. No. 740102: Includes all 13 Capture Beads and all required components		Mix & Match Capture Beads Cat. No.
Target	Bead ID	
GM-CSF	A7	740106
IFN- α	A8	740107
IL-1 α	A5	740104
IL-1 β	A6	740105
IL-11	B6	740113
IL-12 (p40)	B2	740109
IL-12 (p70)	B3	740110
IL-15	B4	740111
IL-18	B5	740112
IL-23	A10	740108
IL-27	B7	740114
IL-33	B9	740115
TSLP	A4	740103
Required Mix and Match Components (already included in Pre-defined Panel)		
Standard		Cat. No. 740117
Detection Antibodies		Cat. No. 740116
Buffer Set (includes Filter Plate)		Cat. No. 740075

Human Proinflammatory Chemokine Panel *(Includes Filter Plate)*

Pre-defined Panel Cat. No. 740003: includes all 13 Capture Beads and all required components		Mix & Match Capture Beads Cat. No.
Target	Bead ID	
CCL2 (MCP-1)	A8	740076
CCL3 (MIP-1 α)	B2	740077
CCL4 (MIP-1 β)	B9	740078
CCL5 (RANTES)	A10	740079
CCL11 (Eotaxin)	A6	740080
CCL17 (TARC)	A7	740081
CCL20 (MIP-3 α)	B5	740082
CXCL1 (GRO α)	B6	740083
CXCL5 (ENA-78)	B4	740084
CXCL8 (IL-8)	A4	740085
CXCL9 (MIG)	B3	740086
CXCL10 (IP-10)	A5	740087
CXCL11 (I-TAC)	B7	740088
Required Mix and Match Components (already included in Pre-defined Panel)		
Standard		Cat. No. 740398
Detection Antibodies		Cat. No. 740072
Buffer Set (includes Filter Plate)		Cat. No. 740075

Human Inflammation Panel *(Includes Filter Plate)*

Pre-defined Panel Cat. No. 740118: Includes all 13 Capture Beads and all required components		Mix & Match Capture Beads Cat. No.
Target	Bead ID	
CCL2 (MCP-1)	A8	740076
IFN- α	A5	740120
IFN- γ	A6	740121
IL-1 β	A4	740119
IL-6	A10	740124
IL-8	B2	740125
IL-10	B3	740126
IL-12 (p70)	B4	740127
IL-17A	B5	740128
IL-18	B6	740129
IL-23	B7	740130
IL-33	B9	740115
TNF- α	A7	740122
Required Mix and Match Components (already included in Pre-defined Panel)		
Standard		Cat. No. 740400
Detection Antibodies		Cat. No. 740132
Buffer Set (includes Filter Plate)		Cat. No. 740075

Human Growth Factor Panel *(Includes Filter Plate)*

Pre-defined Panel Cat. No. 740180: Includes all 13 Capture Beads and all required components		Mix & Match Capture Beads Cat. No.
Target	Bead ID	
Angiopoietin-2	A4	740181
EGF	A5	740182
EPO	A6	740183
FGF-basic	A7	740184
G-CSF	A8	740185
GM-CSF	A10	740186
HGF	B2	740187
M-CSF	B3	740188
PDGF-AA	B4	740189
PDGF-BB	B5	740190
SCF	B6	740191
TGF- α	B7	740192
VEGF	B9	740193
Required Mix and Match Components (already included in Pre-defined Panel)		
Standard		Cat. No. 740194
Detection Antibodies		Cat. No. 740195
Buffer Set (includes Filter Plate)		Cat. No. 740075

Human Adipokine Panel* (Includes Filter Plate)

Pre-defined Panel Cat. No. 740196: includes all 13 Capture Beads and all required components		Mix & Match Capture Beads Cat. No.
Target	Bead ID	
Adiponectin	A4	740197
Adipsin	A5	740198
CCL2 (MCP-1)	A7	740200
CXCL10 (IP-10)	A10	740202
IFN- γ	B6	740207
IL-1 β	A8	740201
IL-6	B5	740206
IL-8	B3	740204
IL-10	B2	740203
Leptin	B4	740205
RBP4	A6	740199
Resistin	B7	740208
TNF- α	B9	740209
Required Mix and Match Components (already included in Pre-defined Panel)		
Standard		Cat. No. 740210
Detection Antibodies		Cat. No. 740211
Buffer Set 3 (includes Filter Plate)		Cat. No. 740215

*The Human Adipokine panel is not suitable for serum/plasma samples due to different sample dilution requirements for different analytes. If you need to measure serum/plasma samples for these analytes, please use Human Metabolic Panel 1 and Human Inflammation panel."

Human CD8/NK Cell Panel (Includes Filter Plate)

Pre-defined Panel Cat. No. 740267: includes all 13 Capture Beads and all required components		Mix & Match Capture Bead Cat. No.
Target	Bead ID	
Granulysin	B9	740280
Granzyme A	B5	740277
Granzyme B	B6	740278
sFas	B2	740274
sFasL	B3	740275
IFN- γ	B4	740276
IL-2	A4	740268
IL-4	A5	740269
IL-6	A7	740044
IL-10	A6	740270
IL-17A	A8	740272
Perforin	B7	740279
TNF- α	A10	740273
Required Mix and Match Components (already included in Pre-defined Panel)		
Standard		Cat. No. 740281
Detection Antibodies		Cat. No. 740282
Buffer Set (includes Filter Plate)		Cat. No. 740075

Human Metabolic Panel 1 (Includes Filter Plate)

Pre-defined Panel Cat. No. 740212: includes all 4 Capture Beads and all required components		Mix & Match Capture Bead Cat. No.
Target	Bead ID	
Adiponectin	A4	740197
Adipsin	A5	740198
Leptin	B4	740205
Resistin	B7	740208
Required Mix and Match Components (already included in Pre-defined Panel)		
Standard		Cat. No. 740213
Detection Antibodies		Cat. No. 740214
Buffer Set 4 (includes Filter Plate)		Cat. No. 740216

Human Anti-Virus Response Panel

Pre-defined Panels include ALL required components and ALL check-marked targets				Mix & Match Capture Bead Cat. No.
Filter Plate		Cat. No. 740349	Cat. No. 740350	
V-bottom Plate		Cat. No. 740390	Cat. No. 740396	
Target	Bead ID	Anti-Virus Response Panel	Type 1/2/3 Interferon Panel	
CXCL10 (IP-10)	A7	✓		740363
GM-CSF	B5	✓		740362
IFN- α 2	B3	✓	✓	740351
IFN- β	B6	✓	✓	740353
IFN- γ	B9	✓	✓	740352
IFN- λ 1	A8	✓	✓	740354
IFN- λ 2/3	B4	✓	✓	740355
IL-1 β	A4	✓		740119
IL-6	A5	✓		740356
IL-8	A10	✓		740357
IL-10	B7	✓		740358
IL-12 (p70)	B2	✓		740361
TNF- α	A6	✓		740359
Required Mix and Match Components (already included in Pre-defined Panel)				
Standard				Cat. No. 740365
Detection Antibodies				Cat. No. 740364
Buffer Set A				Cat. No. 740368
Plate Options				Filter Plate: Cat. No. 740377
				V-bottom Plate: Cat. No. 740379



Mouse Panels

Mouse T Helper Cytokine Panels *(Includes Filter Plate; V-bottom plate version coming soon)*

Pre-defined Panels include ALL required components and ALL check-marked targets										Mix & Match Capture Beads Cat. No.
Cat. No.		740005	740025	740027	740029	740031	740035	740037	740039	
Target	Bead ID	Th	Th1	Th2	Th1/Th2	Th17	Th9	Th22	Tfh	
IFN-γ	A4	✓	✓		✓	✓				740065
IL-2	A7	✓	✓		✓					740054
IL-4	A10	✓		✓	✓					740055
IL-5	A5	✓		✓	✓					740056
IL-6	A8	✓	✓	✓	✓	✓		✓	✓	740057
IL-9	B3	✓					✓			740058
IL-10	B2	✓	✓	✓	✓	✓	✓	✓	✓	740059
IL-13	B9	✓		✓	✓					740060
IL-17A	B4	✓				✓				740061
IL-17F	B5	✓				✓				740062
IL-21	B6	✓				✓			✓	740063
IL-22	B7	✓				✓		✓		740064
TNF-α	A6	✓	✓	✓	✓	✓	✓	✓	✓	740066
Required Mix and Match Components (already included in Pre-defined Panel)										
Standard										Cat. No. 740369
Detection Antibodies										Cat. No. 740073
Buffer Set (includes Filter Plate)										Cat. No. 740075

Mouse Cytokine Panel 2 *(Includes Filter Plate)*

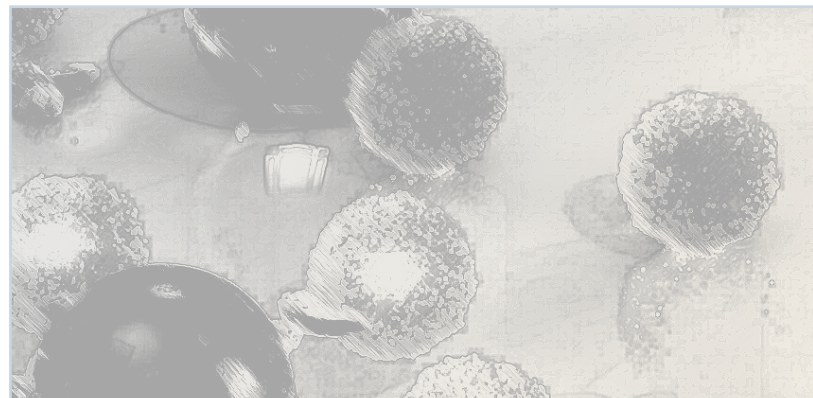
Pre-defined Panel Cat. No. 740134: Includes all 13 Capture Beads and all required components		Mix & Match Capture Beads Cat. No.
Target	Bead ID	
GM-CSF	B7	740146
IFN-β	B6	740145
IL-1α	A4	740135
IL-1β	A5	740136
IL-3	A6	740137
IL-7	B2	740141
IL-11	B3	740142
IL-12 (p40)	A7	740138
IL-12 (p70)	A8	740139
IL-23	A10	740140
IL-27	B4	740143
IL-33	B5	740144
TSLP	B9	740147
Required Mix and Match Components (already included in Pre-defined Panel)		
Standard		Cat. No. 740372
Detection Antibodies		Cat. No. 740149
Buffer Set 2 (includes Filter Plate)		Cat. No. 740166

Mouse Proinflammatory Chemokine *(Includes Filter Plate)*

Pre-defined Panel Cat. No. 740007: Includes all 13 Capture Beads and all required components		Mix & Match Capture Beads Cat. No.
Target	Bead ID	
CCL2 (MCP-1)	A10	740089
CCL3 (MIP-1α)	B4	740090
CCL4 (MIP-1β)	B5	740091
CCL5 (RANTES)	A4	740092
CCL11 (Eotaxin)	A6	740093
CCL17 (TARC)	A7	740094
CCL20 (MIP-3α)	A5	740095
CCL22 (MDC)	B9	740098
CXCL1 (KC)	A8	740096
CXCL5 (LIX)	B7	740097
CXCL9 (MIG)	B2	740099
CXCL10 (IP-10)	B3	740100
CXCL13 (BLC)	B6	740101
Required Mix and Match Components (already included in Pre-defined Panel)		
Standard		Cat. No. 740370
Detection Antibodies		Cat. No. 740074
Buffer Set (includes Filter Plate)		Cat. No. 740075

Mouse Inflammation Panel

Pre-defined Panel includes either V-bottom (Cat. No. 740446) or Filter Plate (Cat. No. 740150), all 13 Capture Beads, and all required components		Mix & Match Capture Beads Cat. No.
Target	Bead ID	
CCL2 (MCP-1)	A8	740155
GM-CSF	B9	740163
IFN-β	B7	740162
IFN-γ	A6	740153
IL-1α	A5	740152
IL-1β	B2	740157
IL-6	B4	740159
IL-10	B3	740158
IL-12 (p70)	A10	740156
IL-17A	B6	740161
IL-23	A4	740151
IL-27	B5	740160
TNF-α	A7	740154
Required Mix and Match Components (already included in Pre-defined Panel)		
Standard		Cat. No. 740371
Detection Antibodies		Cat. No. 740165
Buffer Set B		Cat. No. 740373
Plate Options		Filter Plate: Cat. No. 740377 V-bottom Plate: Cat. No. 740379



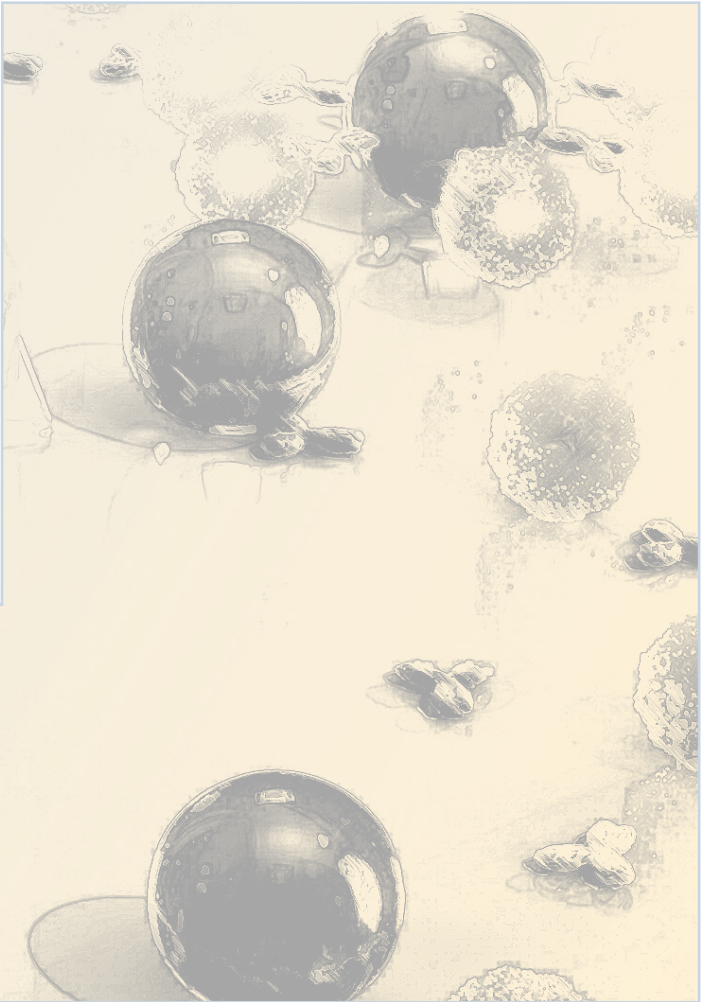
Rat Panels

Rat T helper Cytokine Panels *(Includes either Filter Plate or V-bottom plate)*

Pre-defined Panels include ALL required components and ALL check-marked targets											Mix & Match Capture Beads Cat. No.
Filter Plate Cat. No.		740283	740284	740285	740286	740287	740288	740289	740290	740291	
V-bottom Plate Cat. No.		740402	740403	740404	740405	740406	740407	740408	740409	740410	
Target	Bead ID	Th	Th1	Th2	Th1/Th2	Th17	Th9	Th22	Tfh	ThGM	
GM-CSF	A10	✓	✓	✓	✓	✓				✓	740257
IFN-γ	A5	✓	✓		✓	✓			✓	✓	740253
IL-2	A7	✓	✓		✓					✓	740295
IL-4	B2	✓		✓	✓				✓		740297
IL-5	A6	✓		✓	✓						740294
IL-6	B9	✓	✓	✓	✓	✓		✓	✓		740264
IL-9	B4	✓					✓				740299
IL-10	A4	✓	✓	✓	✓	✓	✓	✓	✓	✓	740252
IL-13	B6	✓		✓	✓			✓			740300
IL-17A	B5	✓				✓					740261
IL-17F	B3	✓				✓					740298
IL-22	B7	✓				✓		✓			740301
TNF-α	A8	✓	✓	✓	✓	✓	✓	✓	✓		740256
Required Mix and Match Components (already included in Pre-defined Panels)											
Standard										Cat. No. 740293	
Detection Antibodies										Cat. No. 740292	
Buffer Set B										Cat. No. 740373	
Plate Options										Filter Plate: Cat. No. 740377	
										V-bottom Plate: Cat. No. 740379	

Rat Inflammation Panel

Pre-defined Panel includes either V-bottom (Cat. No. 740401) or Filter Plate (Cat. No. 740251), all 13 Capture Beads, and all required components		Mix & Match Capture Beads Cat. No.
Target	Bead ID	
CCL2 (MCP-1)	A7	740255
CXCL1 (KC)	A6	740254
GM-CSF	A10	740257
IFN-γ	A5	740253
IL-1α	B7	740263
IL-1β	B4	740260
IL-6	B9	740264
IL-10	A4	740252
IL-12 p70	B3	740259
IL-17A	B5	740261
IL-18	B2	740258
IL-33	B6	740262
TNF-α	A8	740256
Required Mix and Match Components (already included in Pre-defined Panel)		
Standard		Cat. No. 740265
Detection Antibodies		Cat. No. 740266
Buffer Set B		Cat. No. 740373
Plate Options		Filter Plate: Cat. No. 740377
		V-bottom Plate: Cat. No. 740379



Non-Human Primate Panels

Non-Human Primate T Helper Cytokine Panels *(Includes either Filter Plate or V-bottom plate)*

Pre-defined Panels include ALL required components and ALL check-marked targets								Mix & Match Capture Beads Cat. No.
Filter Plate Cat. No.		740303	740381	740382	740383	740384	740385	
V-bottom Plate Cat. No.		740387	740391	740392	740393	740394	740395	
Target	Bead ID	Th	Th1	Th2	Th1/Th2	Th17	Tfh	
IFN- γ	B3	✓	✓		✓	✓	✓	740310
IL-2	A4	✓	✓		✓			740304
IL-4	B4	✓		✓	✓		✓	740311
IL-5	A5	✓		✓	✓			740305
IL-6	A6	✓	✓	✓	✓	✓	✓	740306
IL-10	A7	✓	✓	✓	✓	✓	✓	740307
IL-13	A10	✓		✓	✓			740308
IL-17A	B5	✓				✓		740312
IL-21	B6	✓				✓	✓	740313
TNF- α	B2	✓	✓	✓	✓	✓	✓	740309
Required Mix and Match Components (already included in Pre-defined Panels)								
Standard							Cat. No. 740314	
Detection Antibodies							Cat. No. 740315	
Buffer Set A							Cat. No. 740368	
Plate Options							Filter Plate: Cat. No. 740377	
							V-bottom Plate: Cat. No. 740379	

Non-Human Primate Cytokine/Chemokine Panel

Pre-defined Panel includes either V-bottom (Cat. No. 740388) or Filter Plate (Cat. No. 740317), all 13 Capture Beads, and all required components		Mix & Match Capture Beads Cat. No.
Target	Bead ID	
CCL2 (MCP-1)	A10	740323
CCL3 (MIP-1 α)	B3	740324
CCL4 (MIP-1 β)	B4	740325
CCL5 (RANTES)	B5	740326
CCL11 (Eotaxin)	A6	740320
CXCL9 (MIG)	B2	740348
CXCL10 (IP-10)	A7	740321
CXCL11 (I-TAC)	A8	740322
IFN- γ	B9	740329
IL-1 β	A4	740318
IL-6	A5	740319
IL-8	B6	740327
TNF- α	B7	740328
Required Mix and Match Components (already included in Pre-defined Panel)		
Standard		Cat. No. 740330
Detection Antibodies		Cat. No. 740331
Buffer Set A		Cat. No. 740368
Plate Options		Filter Plate: Cat. No. 740377
		V-bottom Plate: Cat. No. 740379

Non-Human Primate Inflammation Panel

Pre-defined Panel includes either V-bottom (Cat. No. 740389) or Filter Plate (Cat. No. 740332), all 13 Capture Beads, and all required components		Mix & Match Capture Beads Cat. No.
Target	Bead ID	
CCL2 (MCP-1)	B9	740345
CXCL10 (IP-10)	A6	740335
GM-CSF	B6	740343
IFN- β	B2	740339
IFN- γ	B5	740342
IL-1 β	A7	740336
IL-6	A4	740333
IL-8	B7	740344
IL-10	A5	740334
IL-12 (p40)	A8	740337
IL-17A	A10	740338
IL-23	B3	740340
TNF- α	B4	740341
Required Mix and Match Components (already included in Pre-defined Panel)		
Standard		Cat. No. 740346
Detection Antibodies		Cat. No. 740347
Buffer Set A		Cat. No. 740368
Plate Options		Filter Plate: Cat. No. 740377
		V-bottom Plate: Cat. No. 740379



Non-Human Primate Growth Factor Panel

Pre-defined Panel includes either V-bottom (Cat. No. 740386) or Filter Plate (Cat. No. 740217), all 13 Capture Beads, and all required components		Mix & Match Capture Beads Cat. No.
Target	Bead ID	
Angiopoietin-2	A4	740218
EGF	A5	740219
EPO	A6	740220
FGF-basic	A7	740221
G-CSF	A8	740222
GM-CSF	A10	740223
HGF	B2	740224
M-CSF	B3	740225
PDGF-AA	B4	740226
PDGF-BB	B5	740227
SCF	B6	740228
TGF- α	B7	740229
VEGF	B9	740230
Required Mix and Match Components (already included in Pre-defined Panel)		
Standard		Cat. No. 740231
Detection Antibodies		Cat. No. 740232
Buffer Set A		Cat. No. 740368
Plate Options		Filter Plate: Cat. No. 740377
		V-bottom Plate: Cat. No. 740379

Non-Human Primate Adipokine Panel[†] (Includes Filter Plate)

Pre-defined Panel Cat. No. 740233: Includes all 13 Capture Beads and all required components		Mix & Match Capture Beads Cat. No.
Target	Bead ID	
Adiponectin	A4	740234
Adipsin	A5	740235
CCL2 (MCP-1)	A7	740237
CXCL10 (IP-10)	A10	740239
IFN- γ	B6	740243
IL-1 β	A8	740238
IL-6	B5	740242
IL-8	B3	740240
IL-10	B2	740296
Leptin	B4	740241
RBP4	A6	740236
Resistin	B7	740244
TNF- α	B9	740245
Required Mix and Match Components (already included in Pre-defined Panel)		
Standard		Cat. No. 740246
Detection Antibodies		Cat. No. 740247
Buffer Set 3 (includes Filter Plate)		Cat. No. 740215

[†]The NHP Adipokine panel is not suitable for serum/plasma samples due to different sample dilution requirements for different analytes.

Carboxyl Beads (Unconjugated)

Can't find a LEGENDplex™ bead for the target you want? Now you can conjugate your own antibodies to our unconjugated carboxyl beads. Bead IDs are provided so you can fit them appropriately into your LEGENDplex™ panel. If you would like advice regarding the addition of these beads to your panel, talk with us at cst@biolegend.com.

Beads	Cat. No.
A4	740167
A5	740168
A6	740169
A7	740170
A8	740171
A10	740172
B2	740173
B3	740174
B4	740175
B5	740176
B6	740177
B7	740178
B9	740179



Request a Custom LEGENDplex™ panel.

Want a LEGENDplex™ target that you don't see in our listings? Or, do you want to combine cytokines and chemokines across panels? Talk things over with our experts to get your customized project underway.

E-mail: cst@biolegend.com or call 877-BIOLEGEND to get started!



Frequently Asked Questions

What is the difference between LEGENDplex™ and Luminex® assays?

LEGENDplex™ and Luminex® are both bead-based multiplex immunoassays that use the basic principles of sandwich immunoassay principles. Both systems use fluorescence-coded beads to achieve multiplexing capability. The major difference is in the data acquisition. LEGENDplex™ uses common lab flow cytometers for data acquisition, whereas Luminex®-based assays have dedicated machines for this purpose. Therefore, one of the advantages is that LEGENDplex™ can be run on widely available flow cytometers without needing specialized machines. As such, LEGENDplex™ cannot be run on Luminex® machines.

What flow cytometers can run the LEGENDplex™ assays?

LEGENDplex™ assays can be used on most commonly used flow cytometers that can read APC and PE, such as BD FACSCalibur™, BD FACSCanto™, BD FACSCanto™ II, BD LSR I™, BD LSR II™, BD LSRFortessa™, BD FACSAria™ I, II and III, BD Accuri C6™, Beckman Coulter's Gallios™, CytoFLEX™, and Moflo XDP™, Thermo Fisher Scientific's Attune™, ACEA Bioscience's NovoCyte™, EMD Millipore's Guava EasyCyte™. Please refer to the “Materials to be provided by end-user” section of your LEGENDplex™ manual for details on the requirements of the machines in terms of laser and channel configurations. For other brands of flow cytometers, the end-user needs to make sure that the machine is set up properly before use.

BD FACSCalibur™, BD FACSCanto™, BD FACSCanto™ II, BD LSR I™, BD LSR II™, BD LSRFortessa™, BD FACSAria™ I, II and III, BD Accuri C6™, BD FACSVerser™ are trademarks of Becton, Dickinson and Company. Gallios™, CytoFLEX™, and Moflo XDP™ are trademarks of Beckman Coulter, Inc. Attune™ NxT is a trademark of Thermo Fisher Scientific Inc. NovoCyte™ is a trademark of ACEA Biosciences Inc. Guava® easyCyte is a registered trademark of Merck Millipore Corporation. MACSQuant® is a registered trademark of Miltenyi Biotec.

Frequently Asked Questions (Continued)

My standard curve is not linear; can I use the non-linear part of the standard curve during analysis?

Yes. It is possible to use the non-linear part of the standard curve for calculation of results. The LEGENDplex™ data analysis software uses a five-parameter curve-fitting algorithm, which determines the minimum and maximum detection concentrations of each target and reports them. If sample concentrations fall outside of the maximum detectable range, the sample will have to be diluted and reanalyzed.

When do I need to do instrument compensation?

If a single laser instrument is used for both reporter (FL2 or PE) and classification (FL3), then compensation is needed to resolve the signal spillover from FL2 to FL3, and vice-versa. Even on dual laser instruments, some compensation may be required. Please check your machine for the need for compensation and when required, use the compensation beads provided and follow the Flow Cytometer Setup instructions in the LEGENDplex™ manual. In addition, adjust the PMT voltages such that there is good bead separation, the bead populations are not out of scale, and the PE signal is appropriately amplified to ensure proper signal sensitivity.

Is a special software package required for data analysis?

Typically, flow cytometers generate output files in FCS format (e.g. FCS 2.0, 3.0, or 3.1), and in some cases, in list mode file format (LMD). FCS files can be analyzed by different software. Data generated from using LEGENDplex™ kits can be analyzed using the LEGENDplex™ data analysis software freely available for downloading at the BioLegend website ([biolegend.com/legendplex/software](https://www.biolegend.com/legendplex/software)). A free-of-charge software license dongle is provided in the kit (required for PC users). The Mac software is available for use with a file license. Please check our website for most updated versions of the software.

What curve-fitting algorithm can I use for generating my standard curve?

The LEGENDplex™ data analysis software uses a default five-parameter curve-fitting algorithm, which determines the minimum and maximum detection concentrations of each target and reports them. If the default algorithm does not work for a particular set of data, other curve-fitting methods are available in the options of the software. If sample concentrations fall outside of the maximum detectable range, the sample will have to be diluted and re-analyzed.

The kit instruction manual says that the kit is validated for serum, plasma, and culture supernatant sample types. Can I also use tissue homogenates with LEGENDplex™?

Yes, all of the above sample types may be used with LEGENDplex™ kits, as long as they are prepared in a manner compatible with immunoassays. For tissue homogenate samples, they should be prepared in a neutral pH buffer containing no denaturing chemicals (e.g. SDS, urea, thiourea, cholate, etc.). No ionic and a minimal amount of non-ionic detergents (e.g. NP-40) may be used in sample preparation. The tissue/cell homogenization buffer should not contain excessive ionic strength above physiological concentrations of salts. If possible, the buffer should also contain protease inhibitors. In addition, the samples should be centrifuged to remove particulates.

After I finish the assays, how long can I wait before reading my samples (beads) on a flow cytometer?

The plate or tubes containing the bead assays can be re-suspended in Wash Buffer, kept overnight at 4°C while being protected from light, and be read the next day. There may be a decrease in signal, but overall, the assay results should not be significantly affected. Storing the samples for extended periods of time is not recommended, as it could lead to further reductions in signal.

Should I perform the assay with the filter plates or with V-bottom plates?

Filter plates or V-bottom plates have been included in some kits for your convenience. Performing the assay with filter plates is generally recommended. A vacuum filtration unit is required to work with the filter plates. If however, you don't have access to a vacuum manifold, then you can use the V-bottom plates as described in the manual and follow the recommended assay protocols for the type of plates you choose. All plates should be made from low binding polypropylene. Polystyrene ELISA or cell culture plates should not be used.

If I don't have a vacuum, how do I remove the liquid from my plate?

Do NOT invert the plate to dump the liquid. The beads will not adhere to the plate. After centrifugation using a swing bucket rotor with a plate adaptor, you can remove the liquid by using a pipette.

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