TECHNICAL NOTE

Overview of use of the IsoQ Score metric

Automated sample quality metric using IsoQ Score in IsoSpeak software

In this Technical Note we outline:

- The use of the IsoQ Score for potential issues in any run
- How the IsoQ Score can flag potential issues during a run and what these drivers may be
- Methods of analyzing the current issues if any issues arise,
- Troubleshooting to avoid future issues

BRUKER

Single-cell sample quality control using the IsoQ Score test

The single-cell IsoQ Score is a quality metric for each run generated by IsoSpeak Software. Samples are loaded into the program and analyzed for cell quality during this phase.

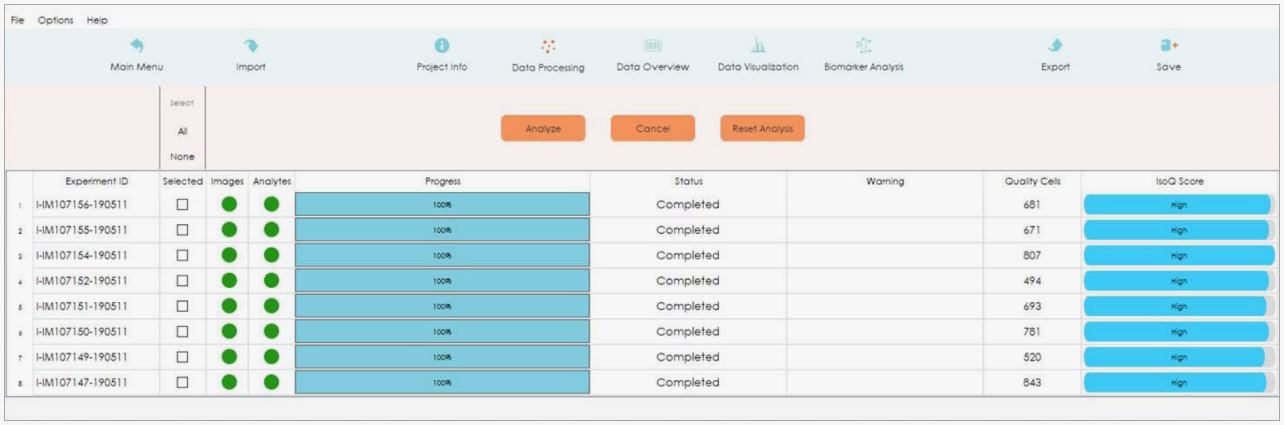
Each sample is then given an IsoQ Score. After the samples are analyzed, they should have a blue IsoQ Score indicating that the sample quality passes and that the data can proceed through the automated IsoSpeak analysis.

What is the IsoQ Score?

The IsoQ score is an assessment of the sample quality determined during the IsoSpeak analysis. The IsoQ score is an algorithm that looks at a combination of sample related elements required to meet specifications, per Bruker protocols, needed to successfully gather single-cell proteomic data. The elements assessed through the IsoQ algorithm include cell number, presence of artifacts in the image and background noise across the signal image, these factors combined can impact the quality and sensitivity of the resulting data. The IsoQ score should be used to judge the success of sample analysis of a given condition.

A high IsoQ Score indicates samples of high quality and absence of interfering artifacts or background noise. A low (Purple) IsoQ score may be an indication of one or more of the following; low cell number that can impact the statistical significance of the data, the presence of artifacts that can impact the quality of the data and or variation in background noise levels across that could affect the sensitivity of the resulting data. A purple IsoQ score is not a conclusive indication that the data is low quality; however, we recommend quality checking this with Bruker to determine if the run is significantly impacted in a way that may affect sample quality.

Single-cell IsoQ Score test interface



	Experiment ID	Selected	Images	Analytes	Progress	Status	Warning	Quality Cells	IsoQ Score
1	HM107156-190511	☐			100%	Completed		681	High
2	HM107155-190511	☐			100%	Completed		671	High
3	HM107154-190511	☐			100%	Completed		807	High
4	HM107152-190511	☐			100%	Completed		494	High
5	HM107151-190511	☐			100%	Completed		693	High
6	HM107150-190511	☐			100%	Completed		781	High
7	HM107149-190511	☐			100%	Completed		520	High
8	HM107147-190511	☐			100%	Completed		843	High

Figure 1 | The single-cell IsoQ Score interface lists each loaded experiment ID (left) and provides the IsoQ Score for the given sample. The status column will indicate when the quality check has been completed and display the number of quality cells and IsoQ Score for each loaded experiment.

Troubleshooting single-cell low sample quality

The components of the IsoQ score correlate to effective sample staining and cell counts, expected background levels and consistency within the signal data, and obtaining accurate and sample-specific data for each measured protein in an automated fashion. If you have a low (purple) IsoQ score you can first assess whether it could be related to cell number by looking at the number of quality cells that were available for analysis in the sample, refer to the "quality Cells" number to determine this. Table 1 provides Troubleshooting recommendations for areas that can contribute to a Purple IsoQ score such as improving low quality cell counts, cell staining and techniques to improve background noise and reduction of debris.

As a general practice, Bruker will assist with troubleshooting all runs with reported purple IsoQ scores. Additionally, Bruker can assist in confirming the accuracy of your single cell data. Users should export a log of the IsoQ scores by going to Export -> IsoQ Score Log and send the log to Bruker customer support for further assistance.

Low single-cell IsoQ Score results interface

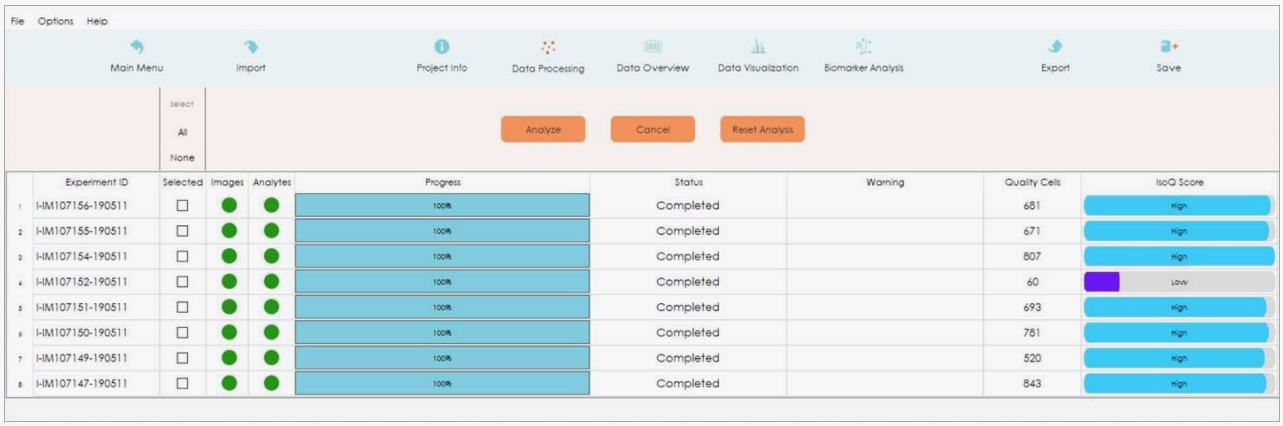


Figure 2 | The single-cell IsoQ Score interface lists each loaded experiment ID (left) and provides the IsoQ Score for the given sample. The status column will indicate when the quality check has been completed and display the number of quality cells and IsoQ Score for each loaded experiment. Purple IsoQ Score results may require additional attention.

Potential sources of artifacts and methods of improving future runs

Contributor to low Q Score	Possible Reason	Recommended Solutions
Low quality cell count on chip ***Cell counting & concentration related***	• Recommended cell concentrations not used • Issue with Cell Counting procedure • Trypan Blue may have debris • Poor cell removal from cell culture plate	• Use recommended cell concentrations during overnight incubation • Use appropriate dilutions recommended • Quick spin Trypan Blue to pellet potential debris, remove aliquot from top of Trypan Blue. • Start with fresh aliquot of Trypan Blue • Thoroughly mix cells in well with pipette prior to transferring to tube
Low quality cell count on chip ***Stain process related***	• Use of media other than the recommended media in protocol which could interact with cell stain • Use of stains not recommended in protocol • Recommended stain concentration, incubation time and/or incubation temperature not used • Cell stain was stored prior to use	• Use complete RPMI media • Use IsoPlexis provided validated stain • Follow recommended staining steps • Use only freshly prepared membrane stain

Low quality cell count on chip
Technique detail related
	• Bubbles loaded onto chip, especially at Chip Loading
	• Detection of potential artifacts such as debris, cell clumping, inefficient enrichment possibly due to:
	  • Pipetting wrong concentration
	  • Not fully inserting column into MACS separator
	  • Reagents not stored at recommended temperatures
	• Recommended number of cells not loaded on chip
		• Avoid introduction of bubbles on chip by mixing and pipetting carefully
		• Ensure use of a sterile space to reduce introduction of potential contaminants.
		• Use dedicated pipettes, tips, and tubes for sterile work. Pipette up and down gently and throughout protocol to reduce clumps.
		• Load recommended number of cells (30,000 cells per chip)

Limited frequency of stimulated cells, i.e., those with cytokine signal ***Viability related***	• Leaving thawed cells in DMSO for an extended period • Low viable cells due to low viability input sample and lack of utilization of Ficoll-Paque • Decreased viability due to cell shock	• After thaw, quickly transfer cells from DMSO to complete RPMI to ensure viability of cells. • Verify viability of cells is above 80% • Use reagents at recommended temperatures (i.e. always use warmed media [37°C])
Limited frequency of stimulated cells, i.e., those with cytokine signal ***Stimulation step related***	• Recommended stimulation concentration was not used • Recommended stimulation duration was not used	• Use IsoPlexis recommended stimulation concentrations • Follow IsoPlexis recommended stimulation times • Use IsoPlexis recommended reagents

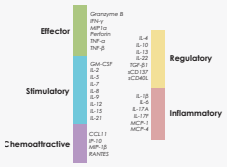
Table 1 | Detailed troubleshooting guides are present in the appendix of every protocol.

Advanced visualization capabilities in IsoSpeak

After IsoSpeak provides the IsoQ Score quality metric, data analysis is automated with the IsoSpeak software pipeline. IsoSpeak can generate a number of advanced visualizations based on the high dimensional functional data. Users can then export visualized datasets to reveal unique single-cell polyfunctional insights, explain critical differences, and uncover mechanisms which can then be fed back into various development and discovery processes to accelerate next generation immune research.

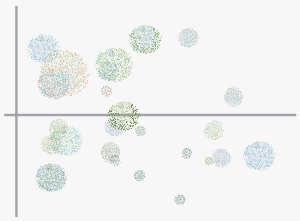
Functional Single-Cell Data Visualizations

32+ functional cytokines per single cell for immune fitness



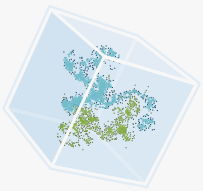
Cytokines define cell function

Stratify donor/patient response by cellular cytokine signature using polyfunctional PCA



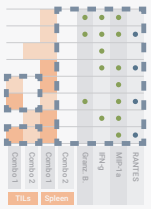
PAT-PCA

Utilize tSNE to differentiate cellular fitness by cytokine subsets



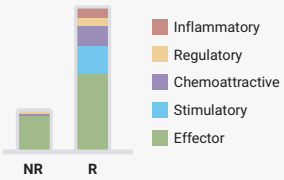
tSNE

Group bioprocessing correlates by potent polyfunctional cells



Heatmap

Discover cell product potency differences with polyfunctional strength (PSI) cytokine signatures



PSI