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Purity control of cells sorted for lineage-specific chimerism analysis.

Allogeneic hematopoietic stem-cell transplantation (HSCT) is used to treat a wide array of hematological disorders. Post-transplantation monitoring is essential to predict negative events, such as disease relapse, graft rejection and graft-versus-host disease. In this context, chimerism analysis is an important method in monitoring post HSCT outcome. Mixed chimerism (the presence of leukocytes from both the donor and the recipient in peripheral blood or in the bone marrow) is mainly evaluated to define engraftment and relapse. Detection of mixed chimerism is a prerequisite in both myeloablative and nonmyeloablative HSCT, in order to assess the graft status and decide later therapeutic strategies.

The clinical importance of chimerism analysis has further increased with the growing use of nonmyeloablative conditioning, which is associated with prolonged duration of mixed hematopoietic chimerism. In addition, the investigation of chimerism within specific leukocyte subsets sorted from peripheral blood or bone marrow samples (by flow-sorting, magnetic beads, or other techniques) provides even more specific information on the graft status, with increased sensitivity.

The purity of the sorted cells can vary greatly depending of the starting cell sample. In particular, pathological samples display often atypical cell proportions. Transportation and storage of patient samples prior to cell sorting can also negatively impact sorting quality. All these factors can result in the presence of contaminating cells in the sorted sample, i.e. cells of another cell type than the one desired. Flow cytometry is classically used to assess cell purity, but this technique is not readily accessible to all laboratories. In addition, the cells have to be run on the flow cytometer shortly after cell sorting; they cannot be easily stored for extended periods of time. And the number of cells available post-sorting can be very low, especially with pediatric or leukopenic samples. In practice, because access to a flow cytometer is limited, or because not enough cells can be spared for flow cytometry analysis, a large proportion of chimerism laboratories do not perform routine purity check after cell isolation.

Knowing the purity of the sorted cells is an essential quality control step, as stated in the EFI and ASHI guidelines*. Contamination of Lymphocytes preparations by Myeloid cells is a frequent issue during cell sorting and, if not documented, can significantly impact the accuracy and reliability of lineage-specific chimerism analysis.

Accumol's Non-T and Non-B Genomic Detection Kits are designed to address these problems, and allow the easy integration of the purity control step in the chimerism analysis workflow.

These kits detect the presence of contaminating cells in T or B Cell preparations, at the level of the genomic DNA. They are used in place of flow cytometry to assess T or B Cell purity.

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The test is performed using about 2 ng of genomic DNA, from the same sample that is used for the chimerism analysis. Therefore, no extra cells need to be set aside for the assay, and the amount of DNA used is only a fraction of the quantity usually recovered during standard DNA isolation procedures. This makes the purity control possible on samples containing very few cells. Indeed, 2 ng of DNA can be recovered from approximately 1000 cells.

Since the tests are performed on DNA, the kits are not affected by the presence of beads or antibodies on the surface of the sorted cells, and can be performed regardless of the sorting method used (flow cytometry, magnetic beads, density-based, etc...). And because the kits detect the purity information directly at the level of the DNA sample, the sample can be analyzed at any point in time after DNA isolation, even years after the actual cell sorting took place.

In addition, chimerism tests are based on DNA analysis, and so are the Non-T Genomic and Non-B Genomic Detection Kits. All the DNA present in the sample will be taken into account when calculating purity. In contrast, flow cytometry analysis often excludes "debris" and events that have a small size. These may contain DNA fragments or even entire nuclei. This DNA is excluded from the flow cytometry data, but might have an impact on the chimerism analysis results.

***EFI Standards, Section I4.190:**

"When HCE (Haemopoietic Chimaerism and Engraftment) testing is performed on cellular subsets isolated by cell sorting, the purity of the sorted population must be documented and taken into account in the analysis of the results. If this is not possible it must be clearly stated in the report."

ASHI Guidelines, Section D.5.3.4.1.8:

"Document the purity obtained if processing involves isolation of cell subsets. If purity is not assessed, this must be documented on the test report."

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Non-T Genomic Detection Kit

ACCUMOL's Non-T Genomic Detection Kit allows the detection of Non-T Cell DNA in genomic DNA samples prepared from sorted T Cells. During normal T Cell differentiation, rearrangement of the TCR genes results in the excision of genomic DNA. This modification of the genome takes place on both alleles. The Non-T Genomic Detection Kit is designed to amplify a sequence located in the excised region, that is no longer part of the genome of mature T Cells. After PCR amplification, the reaction products are analyzed by fragment analysis on a sequencer. Alternatively, analysis can be performed on agarose or polyacrylamide gel. The detection of the target sequence in the gDNA sample indicates that contaminating Non-T Cells were present in the original T Cell preparation.

Since the test is performed on DNA, ACCUMOL's Non-T Genomic Detection Kit is not affected by the presence of beads or antibodies on the surface of the sorted cells, and can be performed regardless of the sorting method used (flow cytometry, magnetic beads, density-based, adhesion, etc...). And because ACCUMOL's Non-T Genomic Detection Kit detects the purity information directly at the level of the DNA sample, the sample can be analyzed at any point in time, even years after the actual cell sorting took place.

Easy to use

The Non-T Genomic Detection Kit is provided ready to use as a reaction mix aliquoted in 0.2 mL PCR tubes. Just add the template (between 0.5 and 10 ng of gDNA), and load the reaction tube in your thermocycler. The PCR program we recommend is a standard 30 cycles 3-step program. The Non-T Genomic Detection Kit is very robust and will perform under a wide range of PCR conditions.

Note that this kit has been primarily designed to integrate seamlessly in chimerism analysis workflows. PCR programs used in most chimerism analysis protocols are compatible with the Non-T Genomic Detection Kit. If you are performing chimerism analysis, you will likely not need to re-program your thermocycler. The reaction can be run along with your chimerism samples, with your existing settings.

Similarly, PCR products analysis is preferentially performed on a sequencer, using conditions similar to the ones used in most chimerism analysis procedures.

Alternatively, PCR products can be analyzed by electrophoresis on agarose or polyacrylamide gel. However, the sensitivity and dynamic range will be significantly reduced as compared to fragment analysis on a sequencer.

Cat#: PB-010013 (32 reactions)

Cat#: PB-010012 (96 reactions)

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Non-B Genomic Detection Kit

ACCUMOL's Non-B Genomic Detection Kit allows the detection of Non-B Cell DNA in genomic DNA samples prepared from sorted B Cells. During normal B Cell differentiation, rearrangement of the IGH genes results in the excision of genomic DNA. This modification of the genome takes place on both alleles. The Non-B Genomic Detection Kit is designed to amplify a sequence located in the excised region, that is no longer part of the genome of mature B Cells. After PCR amplification, the reaction products are analyzed by fragment analysis on a sequencer. Alternatively, analysis can be performed on agarose or polyacrylamide gel. The detection of the target sequence in the gDNA sample indicates that contaminating Non-B Cells were present in the original B Cell preparation.

Since the test is performed on DNA, ACCUMOL's Non-B Genomic Detection Kit is not affected by the presence of beads or antibodies on the surface of the sorted cells, and can be performed regardless of the sorting method used (flow cytometry, magnetic beads, density-based, adhesion, etc...). And because ACCUMOL's Non-B Genomic Detection Kit detects the purity information directly at the level of the DNA sample, the sample can be analyzed at any point in time, even years after the actual cell sorting took place.

Easy to use

The Non-B Genomic Detection Kit is provided ready to use as a reaction mix aliquoted in 0.2 mL PCR tubes. Just add the template (between 0.5 and 10 ng of gDNA), and load the reaction tube in your thermocycler. The PCR program we recommend is a standard 30 cycles 3-step program. The Non-B Genomic Detection Kit is very robust and will perform under a wide range of PCR conditions.

Note that this kit has been primarily designed to integrate seamlessly in chimerism analysis workflows. PCR programs used in most chimerism analysis protocols are compatible with the Non-B Genomic Detection Kit. If you are performing chimerism analysis, you will likely not need to re-program your thermocycler. The reaction can be run along with your chimerism samples, with your existing settings.

Similarly, PCR products analysis is preferentially performed on a sequencer, using conditions similar to the ones used in most chimerism analysis procedures.

Alternatively, PCR products can be analyzed by electrophoresis on agarose or polyacrylamide gel. However, the sensitivity and dynamic range will be significantly reduced as compared to fragment analysis on a sequencer.

Cat#: PB-010023 (32 reactions)

Cat#: PB-010022 (96 reactions)