Quick-Start Protocol September 2016

QIAscout™ 12,000-Microraft Arrays

QIAscout 12,000-Microraft Arrays (cat. no. 928031) are meant for use only in conjunction with the QIAscout (cat. no. 9002733). The arrays are shipped at room temperature. Upon receipt, store the arrays at room temperature (15—25°C) in a dry location. This protocol is for the preparation of the QIAscout array and seeding of cells prior to isolation of single cells using the QIAscout instrument.

Further information

- *QIAscout User Manual*: **www.qiagen.com/QIAscout**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Seeding and cultivation of cells on the QIAscout array is comparable to standard cell culture vessels. Adherent cells are plated on the QIAscout array in the same manner as one would on a standard tissue culture dish, whereas suspension cells require an extra coating of the QIAscout array. Prepare a single-cell suspension following the conventional method for the cell line being used and determine the cell density using a standard cell counting method.

The cell distribution on the QIAscout array follows a Poisson distribution (Table 1). To achieve the maximum number of microrafts on the QIAscout array containing a single cell and to minimize the number of microrafts with no cell or more than one cell, it is suggested to plate cells at a cell : microraft ratio of 1:2 or 1:3 (as highlighted in Table 1). A QIAscout array contains 12,000 individual microrafts, and therefore plating 4,000 – 6,000 cells is generally recommended. Adjust the cell density of your suspension and add the desired number of cells to the QIAscout array in 2 ml of medium.

Table 1. Cell distribution on the array follows a Poisson distribution

Cell : Well ratio	%Empty wells	%Wells capturing a single cell	%Wells capturing ≥ 2 cells
1:1	37	37	26
1:2	**61**	**30**	**9**
1:3	**72**	**24**	**4**
1:5	82	16	2
1:10	90	9	1

After adding cells to the QIAscout array, allow the cells to settle and attach firmly enough to the microraft surface before proceeding for release and collection. A waiting time of 6–10 hours in culture is generally adequate, but the length of this period will be cell line-dependent.

Once settled, cells cultured on the QIAscout array remain localized on the microraft surface, and migration across the wall to adjacent microrafts rarely occurs, the exception being when the cells reach confluence on the microrafts. The time window for cell isolation can be anywhere from a few hours to several days after cell plating. Several days of expansion is suitable if the objective is to isolate clonal colonies using QIAscout arrays.

Important points before starting

- It is strongly recommended not to pierce the outer 7 lines of the QIAscout array, as the microrafts included in these lines are not made pierceable by the manufacturing process.
- For orientation on the array, microrafts are numbered in their x- and y-coordinates. This facilitates choosing of a microraft containing the cell of interest (see figure 1A-B). Please refer to the *QIAscout User Manual* for more related information.

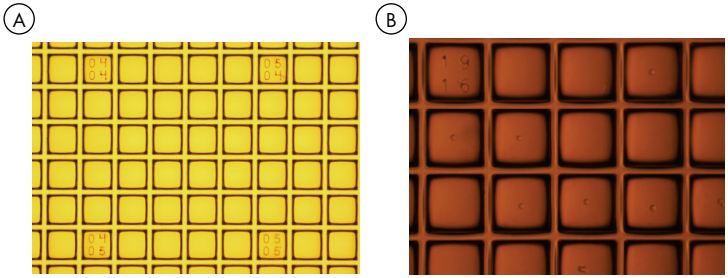

Figure 1. A. Numbered microrafts on the QIAscout array. B. Microrafts containing single cells.

Protocol

Preparation of the QIAscout array and seeding of cells

1. In a sterile cell culture hood, remove the QIAscout array from the sealed pouch.

   Note: The QIAscout array might have a damp appearance in some areas, which however does not affect the quality and usability of the array.

2. Remove the lid and add 2 ml of sterile PBS to the QIAscout array.

3. Wait at least 3 minutes, and then remove the PBS.

   Note: The QIAscout array is covered with a proprietary water-soluble biocompatible coating that effectively prevents air bubble formation when adding liquid to the array. These wash steps ensure the complete removal of this coating. Do not allow the QIAscout array to dry during preparation. This will cause air bubbles to form that will become trapped in the microrafts.

4. Repeat steps 2–3.

5. If working with suspension cells or with cells that require an extracellular matrix (ECM) for culture (such as gelatin, collagen, fibronectin, matrigel, etc.), coat the QIAscout array using standard coating protocols in the same manner as one would coat standard cell culture dishes. For suspension cells it is recommended to use an adhesive solution that immobilizes the cells (e.g., Corning® Cell-Tak™).

If the cells do not require any coating, proceed to step 6.

Note: Ensure that the QIAscout array does not become dry during the coating process.

6. Repeat steps 2–3 with cell culture medium instead of sterile PBS.
7. Generate a single-cell suspension of 4,000 – 6,000 cells in 2 ml of desired culture medium.
8. Add cells in suspension evenly across the entire QIAscout array.

   Note: It is also possible to first add 2 ml medium to the QIAscout array and then add the cells suspended in a smaller volume. When using this method, it is recommended to swirl the QIAscout array in the pattern of '8' in order to achieve an equal distribution of cells on the array.
9. Cultivate cells under the standard culture conditions for the cell line being used.

   Note: Depending on the cell line used, cells will usually attach within 6–10 hours to the QIAscout array.
10. Once adhered, screen the QIAscout array using brightfield, fluorescence or confocal microscopy to identify target cells.

Ordering Information

Product	Contents	Cat. no.
QIAscout 12,000-Microraft Arrays	5 arrays	928031

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.

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