| | | | |
|---|---|---|---|
| **Title of Qualification:** | **Qualification of Aquaguard-1** | | |
| **Test Material Name and Lot #** | Aquaguard-1 lot 1546533 | | |
| **Control Material Name and Lot #** | Incubator chamber with standard DI water | | |
| **Cell Culture Medium Used** | Medium Name: mTeSR1 | | |
| | Component | Manufacturer | Lot # |
| | Basal Medium | STEMCELL Technologies | 15J66194 |
| | 5X Supplement | STEMCELL Technologies | 15J66193 |
| | 250X Growth Factor without bFGF | STEMCELL Technologies | 15J66192 |
| | bFGF | Waisman | WC-FGF2-FP-004 |
| **Platform/Matrix** (MEFs, matrigel, etc) | Matrigel | | |
| **Technician** | | | |
| **Start and End Dates of Qualification** | 22Jun16 and 22Jul16 | | |
| **PSC line, lot, and thaw used** | WIC-WA09-RB-001-T34976 | | |
| **Pre- Karyotype** (enter "Normal" or "Abnormal" and the sample #) | Normal | Karyotype Sample #: 11701 | |
| **Post-Karyotype** (enter "Normal" or "Abnormal" and the sample #) | Normal | Karyotype Sample #: 11754 | |
| **QC Qualification Sample ID** | 11718 | | |

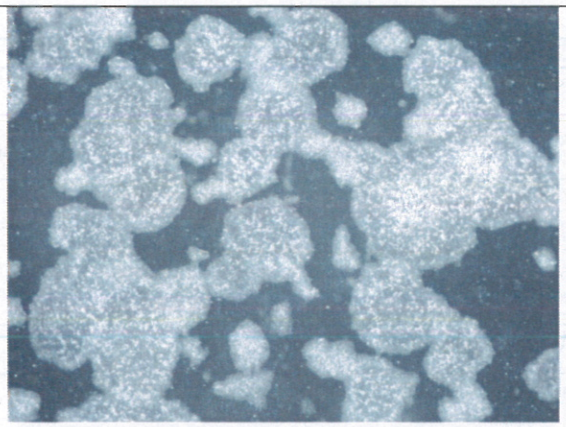
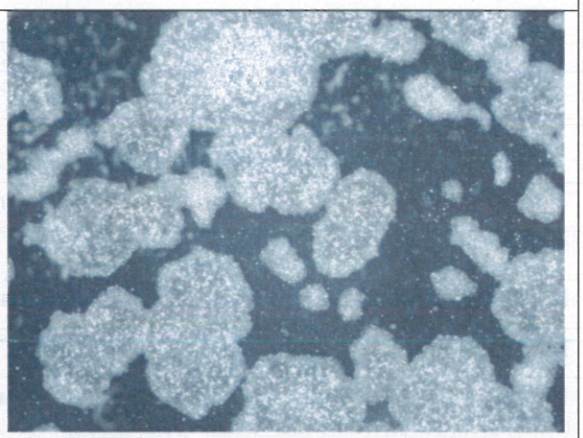
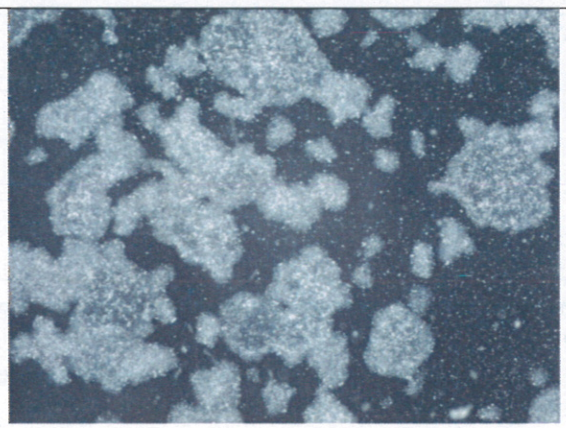
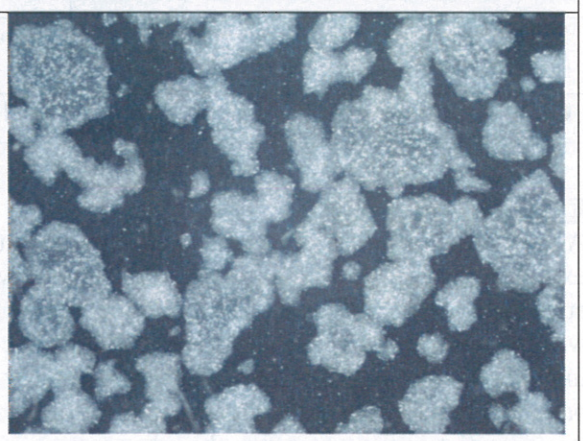
**Experimental design:**

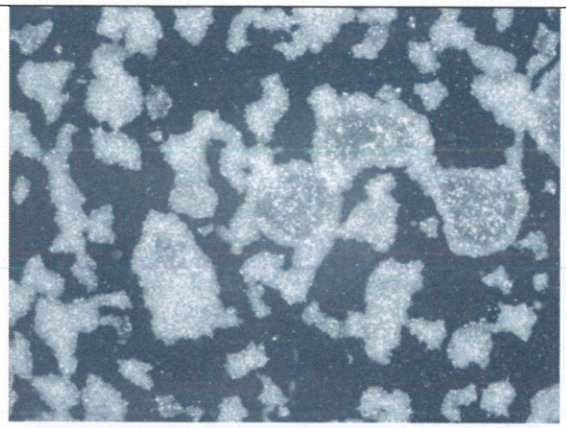
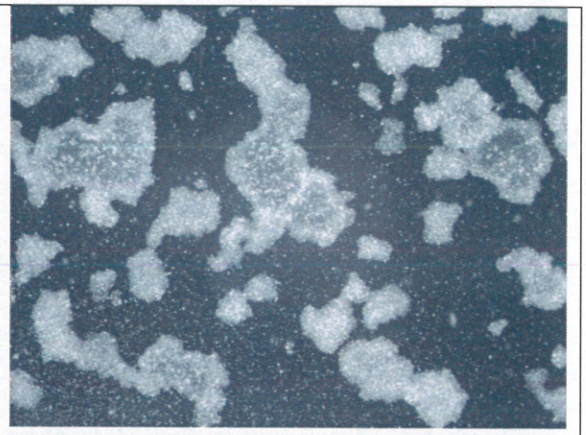
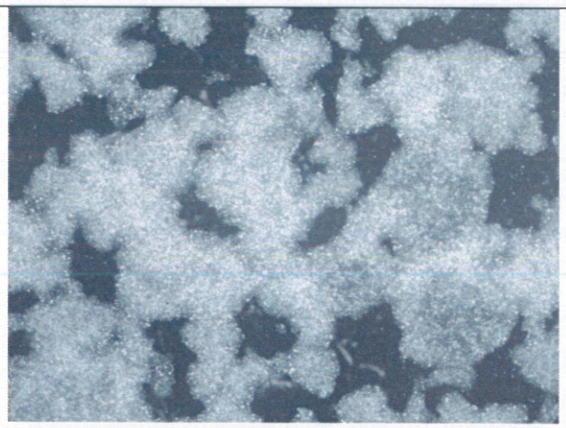
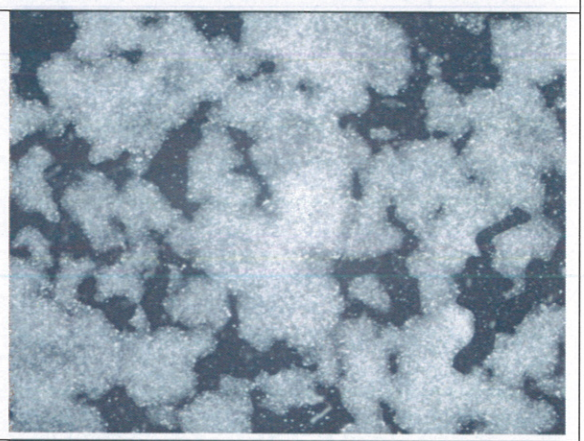
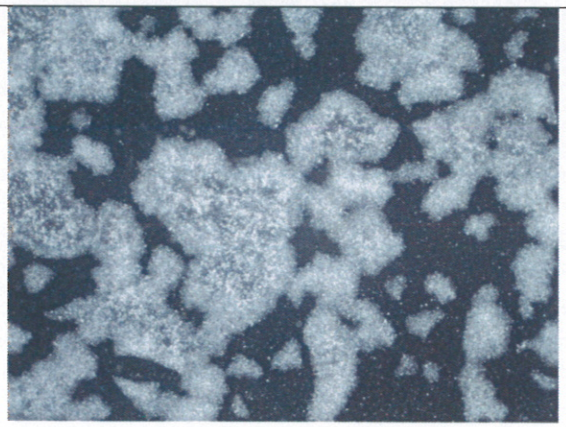
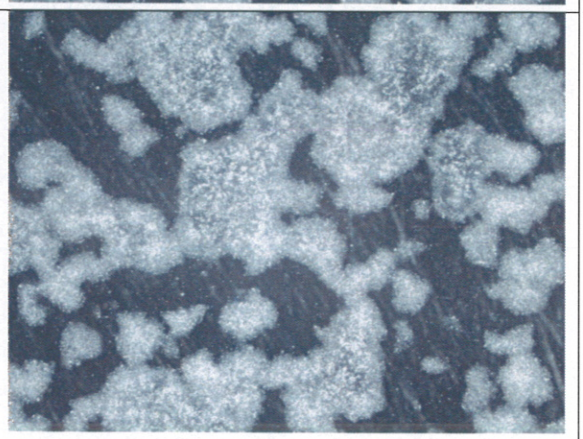
Aquaguard-1 lot 1546533 was tested for the potential to effect the undifferentiated status and the expansion rate of PSCs when used in the humidity pan of a properly maintained incubator (parameters set at 37°C, 5% $CO_2$, 20% $O_2$). The water pan of one incubator chamber (containing 4L of filtered DI water) was treated with 40ml of Aquaguard-1 (final concentration = 1%) at the initiation of the study, and again in 2-week intervals following initiation. Recently karyotyped cells originating from a single culture were passaged into two separate cultures; one culture was maintained in the incubator chamber with Aquaguard-1 in the water pan, the other culture was maintained in a chamber without Aquaguard-1 in the water pan – all other culture parameters including medium, matrix, gas atmosphere, temperature, technician, processing times, etc., remained the same between the cultures. Cultures were maintained for 5 passages in each respective chamber. Following 5 passages, the resulting cultures were submitted for karyotype, assayed via flow cytometry to determine the percent of undifferentiated cells in culture, assessed for overall expansion rate, and morphology was examined. Testing was performed per WiCell's SOP-QU-005-F, Quality Control Testing of Cell Culture Reagents. Documentation was recorded in notebook 184 pages 158-169 and notebook 187 pages 71-86.

**Equipment:**

| | | |
|---|---|---|
| BioSafety Cabinet | 3-digit equipment ID: 094 | Room #: 119 |
| Incubator | 3-digit equipment ID: 125 | Room #: 115 |
| Microscope | 3-digit equipment ID: 194 | Room #: 119 |
| Micropipettor | S/N: Y60607A | Room #: 119 |

**Images of PSCs just prior to passaging at 2x magnification:**

| | Control Material | Test Material |
|---|---|---|
| P1 |  |  |
| P2 |  |  |

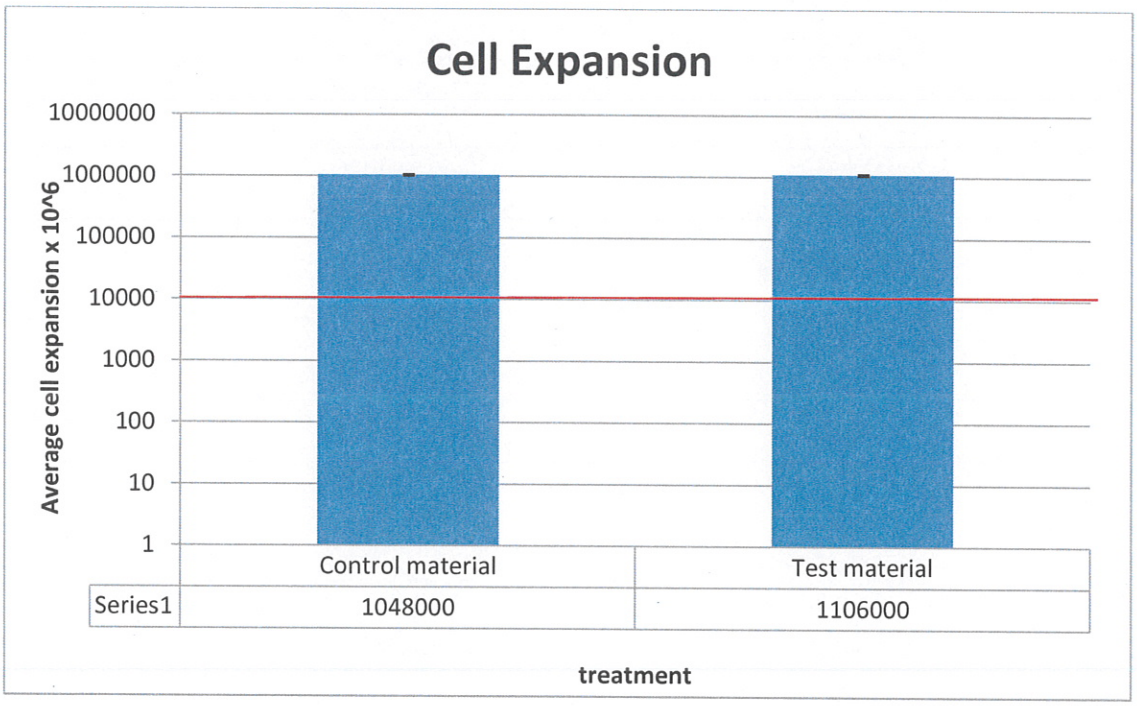
| | Control Material | Test Material |
|---|---|---|
| P3 |  |  |
| P4 |  |  |
| P5 |  |  |

**Proliferation Data (Split Ratios and Cell Counts):**

Minimum Acceptable Expansion Rates:

*Cultures passaged with EDTA*: Average rate of 1:10 and at least 1 million cells/well at final count. Virtual expansion per well to equal 10000 wells at 1 million cells/well = 10000 million cells.

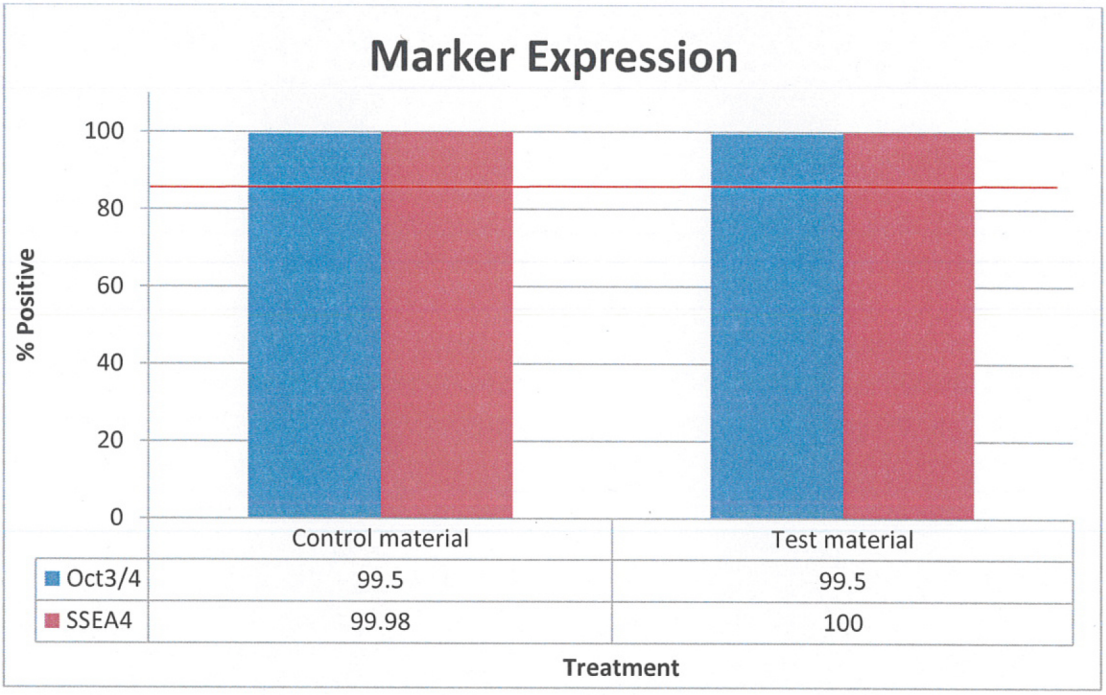
| Virtual Expansion Information Table | | |
|---|---|---|
| Passage # | Control Material Split Ratio 1:X | Test Material Split Ratio 1:X |
| 1 to 2 | 20 | 20 |
| 2 to 3 | 20 | 20 |
| 3 to 4 | 20 | 20 |
| 4 to 5 | 25 | 25 |
| Total Virtual Wells | 200000 | 200000 |
| Average Viable Cells / Well x$10^6$ | 5.24 | 5.53 |
| Virtual Expansion x $10^6$ cells | 1048000 | 1106000 |




Red line indicates minimum acceptable expansion.

**Marker Expression:**

Minimal expression acceptable: ≥ 85% positive for Oct3/4 and SSEA4 markers for undifferentiated PSCs.




Red line indicates minimum acceptable marker expression.

**Conclusions:**

No effect on cell proliferation, differentiation, morphology or karyotype was noted for human pluripotent cells cultured in the presence of 1% Aquaguard-1 solution (lot 1546533) in the water pan of a properly maintained incubator (parameters set at 37°C, 5% $CO_2$, 20% $O_2$). Cells cultured in the presence of 1% Aquaguard-1 solution in the water/humidity pan met all WiCell requirements for quality. Aquaguard-1 equivalent to lot 1546533, when used as directed, is appropriate for use in pluripotent cell culture.

**Technician Signature:** _____ **Date:** 16Aug16

**Reviewer Signature:** _____ **Date:** 15AUG16

**QA Signature:** _____ **Date:** 16Aug16