CHAO FAMILY COMPREHENSIVE CANCER CENTER
UNIVERSITY of CALIFORNIA, IRVINE

EXPERIMENT RESULT of Puriton / Cell Viability Assay

Company: Kadesh

Sample: Puriton (lot# CP-021917)

Report Date: 06-23-2017

1. Cell Viability Assay (ATP Luminescent Assay)

The Luminescent Cell Viability Assay is a homogeneous method to determine the number of viable cells in culture based on quantitation of the ATP present, which signals the presence of metabolically active cells. Total levels of cellular ATP can be used to assess cell viability, cell proliferation and cytotoxicity of a wide range of compounds and biological response modifiers.

2. Cell Line : Melanoma (A375), Renal Cancer (RCC4(-), PC3, HL60

3. Method : Luminescent cell viability assay

At 24 h post-subculture, the cells in the 96-well plate were treated with Puritan, H2O and H20-pH12 for 1 days. Cellular viability was measured using the CellTiter-Glo® Luminescent Cell Viability assay (Promega Corporation, Madison, WI, USA). The 96-well plate was briefly equilibrated to room temperature for ~30 min. The CellTiter-Glo® reagent (100 µl) was then added to each well. Following this, the media and reagent were mixed for 2 min on an orbital shaker and left to incubate at room temperature for 10 min prior to recording luminescence, using a Tecan Infinite F200® microplate reader . Luminescence final values were presented as a relative percentage.

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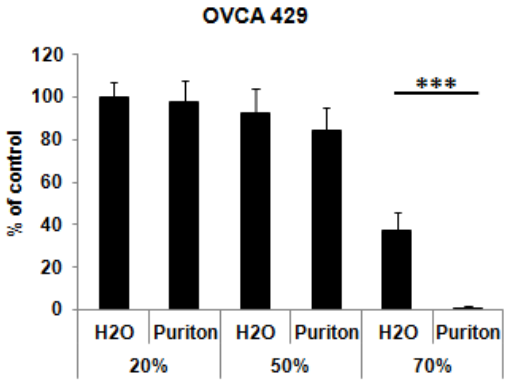
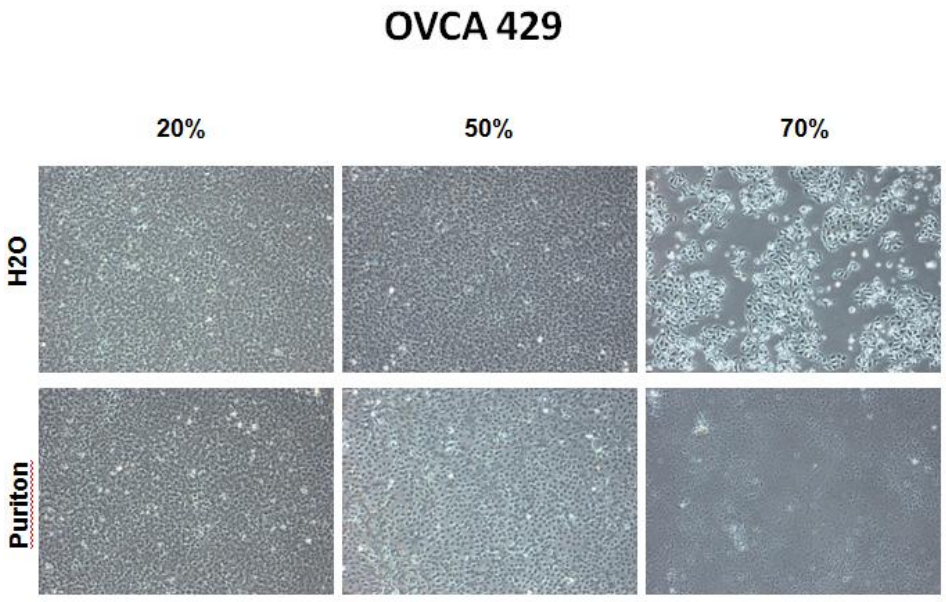
1. Prostate Cancer Cell

1) Prostate Cancer Cell (전립성 암): **PC3**
2) Reagent and treatment (Day 1) : Puriton, H2O
3) Cell Viability Assay (ATP Luminescent Assay)

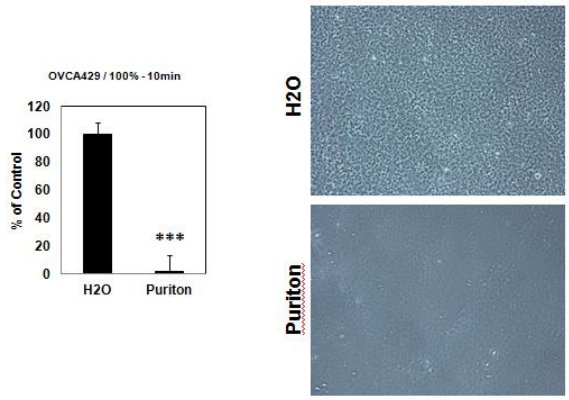
OVCA 429 (Ovary Cancer Cell)



**p<.01, ***p<.005 compared with the control (H2O).



OVCA 429 (Ovary Cancer Cell) / 100% treat - 10min



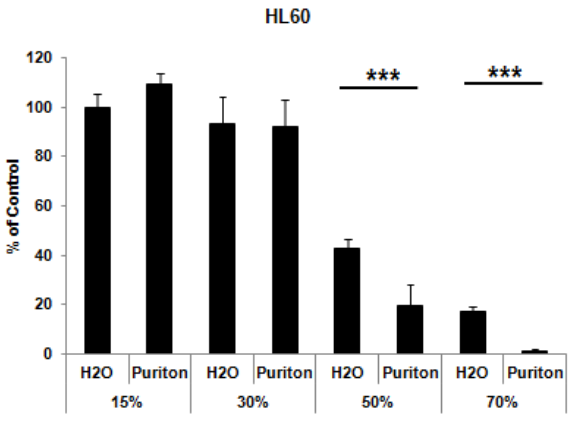
**p<.01, ***p<.005 compared with the control (H2O).

2. Promyelocytic Leukemia

1) Promyelocytic Leukemia (혈액암, 백혈병): **HL60**
2) Reagent and treatment (Day 2) : Puriton, H2O
3) Cell Viability Assay (Tryphan Blue)

HL60 / 2day /Tryphan Blue

HL60 (Promyelocytic Leukemia)

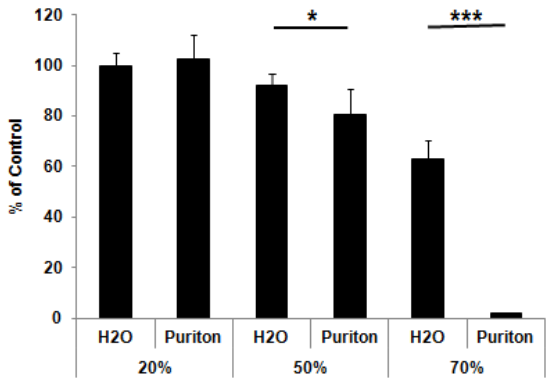


**p<.01, ***p<.005 compared with the control (H2O).

3. Melanoma Cell

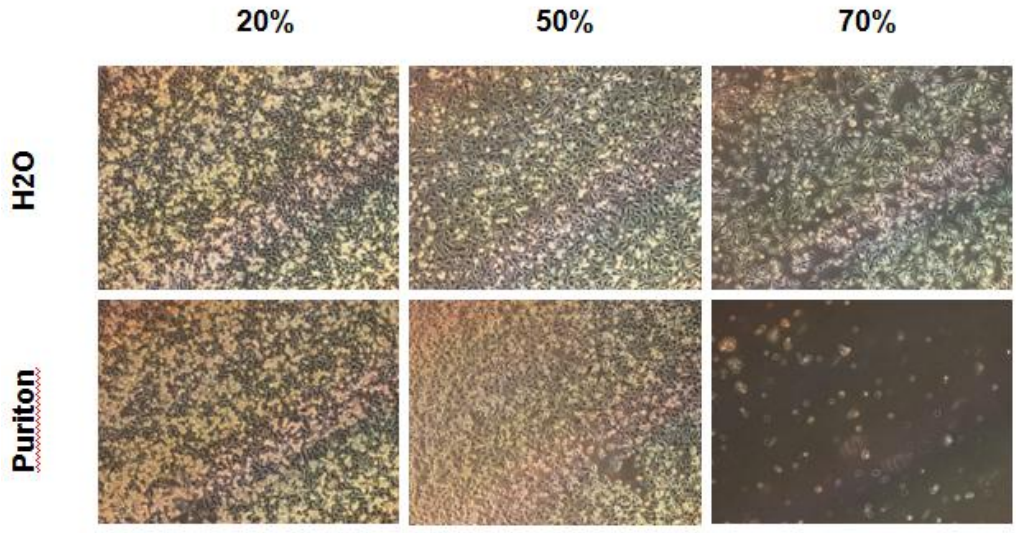
1) Melanoma Cell (흑색종, 피부암): **A375**
2) Reagent and treatment (Day 2) : Puriton, H2O
3) Cell Viability Assay (ATP Luminescent Assay)

Melanoma (A375)



* p<.05, ** p<.01, *** p<.005 compared with the control (H2O).

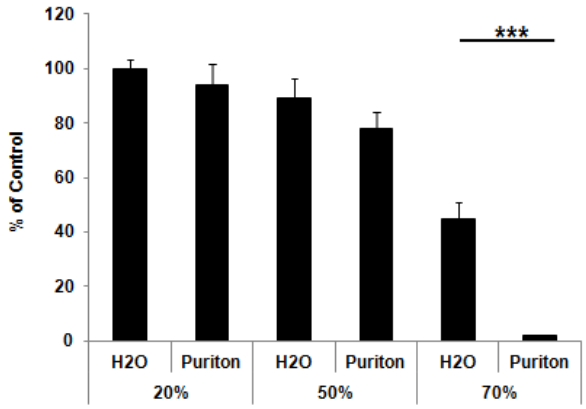
Melanoma (A375)



4. Renal Cancer Cell

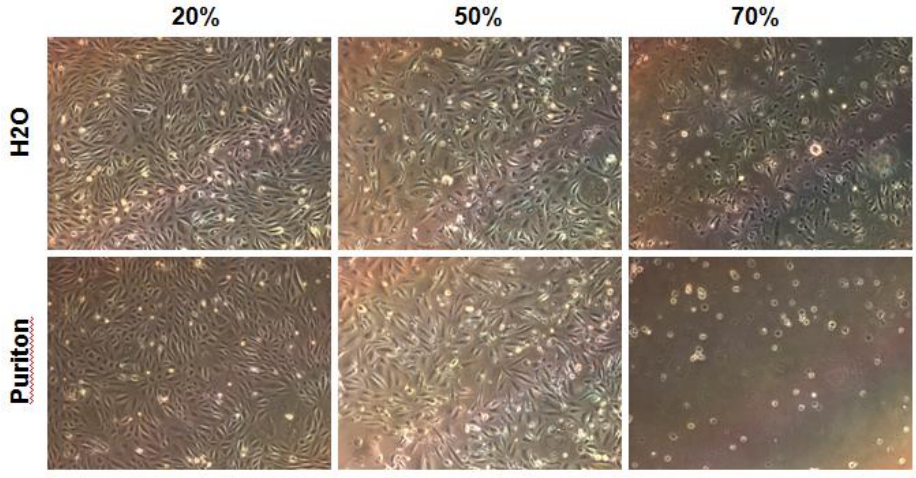
1) Renal Cancer Cell (신장암): **RCC4(-)**
2) Reagent and treatment (Day 2) : Puriton, H2O
3) Cell Viability Assay (ATP Luminescent Assay)

Renal Cancer Cell: RCC4(-)



* p<.05, ** p<.01, *** p<.005 compared with the control (H2O).

Renal Cancer Cell: RCC4(-)



5. Summary

1) Puriton has 40% of cell proliferation inhibition of PC3 cells compare to H2O at 50 % treatment. At 70% Puriton treatment to PC3, Puriton has 0% cell survival.
2) Purion at 30% treatment for 3 days has 5-10% PC3 cell viability.
3) Purion at 70% treatment for 3 days has 99% OVCA429 cell growth inhibition.
4) Purion has 70% and 99% HL60 cell growth inhibition respectively compare to H2O at 50% and 70% treatment for 3 days.
5) Purion has 20% and 99% A375 cell growth inhibition compare to H2O at 50% and 70% treatment for 2 days.
6) Purion has 90% RCC (-), renal cancer cell growth inhibition compare to H2O at 70% treatment for 2 days.