NEUROMICS

# Human Prostate Carcinoma Cells (DU 145)

**Catalog #:** DU001

**Cell #:** >5x10$^5$ cells

**Storage:** Liquid Nitrogen until ready for culture.
While Culturing keep in 37°C $CO_2$ incubator

**Product Format:** Frozen Vial

## GENERAL INFORMATION

Human Prostate Carcinoma Cells (DU 145) were derived from a prostate carcinoma patient. They were taken from a metastatic site in the brain of a 69 year old Caucasian male. DU 145 are not hormone-sensitive and do not express prostate-specific antigen (PSA). Cells are supplied in frozen vials with more than 5x10^5 cells/vial. Universal Full Growth Medium (TM001) is recommended to culture the cells.

*Product is for Research use only.*

Frozen Vials are shipped in a Dry Ice Package.

## CHARACTERIZATION OF THE CELLS

Human Prostate Carcinoma Cells (DU 145) are tested negative for HIV-1, HBV, HCV, and mycoplasma.

## HANDLING OF ARRIVING CELLS

When you receive the dry ice package with cells in frozen vials, transfer the frozen vials of cells into a -80ºC freezer for short period storage or a liquid nitrogen tank for long-term storage.

## PROTOCOL FOR THAWING THE CELLS AND SUBCULTURE

**Note:** If you have any questions or need clarification regarding the protocol for culturing these cells, please reach out to Dr. Jensen Auguste at (978) 608-1766 with your questions before beginning.

1. Pre-coating of T25 flasks: Add 2 ml of Universal Coating Solution (AC002) into a T25 flask to cover the whole surface of the flask, 5 mins later, dispose the excessive coating solution by aspiration. The flask is ready to be used.

2. Thaw the frozen cell vial in a 37ºC water bath first, and then transfer the cells into the pre-coated T25 flask with 10 ml of Universal Full Growth Medium (TM001), cells usually become confluent with 5-7 days.

3. To passage the cells, rinse the cells in a T25 flask with 5 ml HBSS (RT) twice; then add 2 ml Universal Detachment Solution (RT) (AD002) into one T25 flask; gently dispose the excessive Universal Detachment Solution within 20 seconds by aspiration.

4. Leave the T25 flask with the cells at RT or 37ºC for 1 min (most cells usually will detach from the surface within 1-2 mins) or monitor the cells under a microscope until most of cells become rounded up, and then gently tap the flask against the bench surface, and the cells will move on the surface of the flask when monitoring under microscope.



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5. Add 5 ml of Universal Neutralization Buffer (NB001) and spin down the cells with an 800 g centrifugation for 5 mins.

6. Re-suspend the cell pellet with 10 ml or 15 ml of Universal Full Growth Medium and transfer 5 ml each into 2 or 3 pre-coated T25 flasks (for 1/2 to 1/3 subculture ratio).

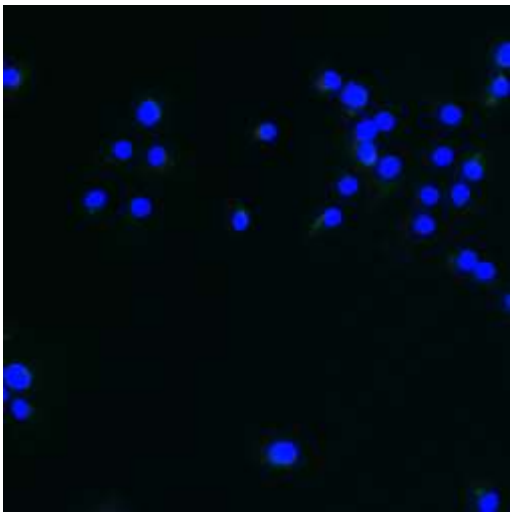
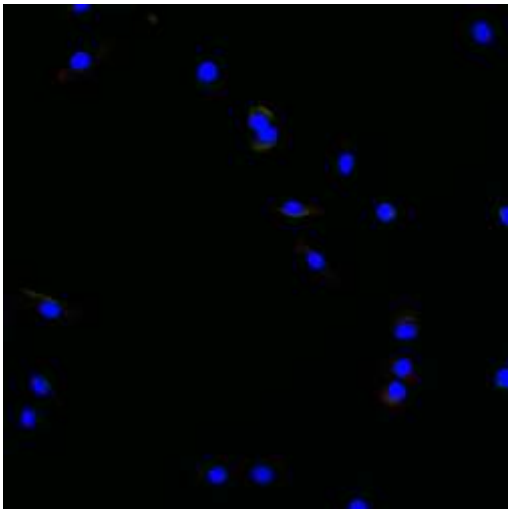
7. Change the medium every 2 or 3 days and the cells usually become confluent within 7 days (when split at a 1/3 ratio).

## KRT18 and KRT 19 Staining of DU145

Control: Fixed cells were incubated with only a mixture of anti-mouse and anti-rabbit fluorescent secondary antibodies to ensure there is no specific staining. Which none was seen.

Experimental wells: Fixed cells were stained with both KRT19 and KRT 18. The co-localization of KRT18 with KRT19 indicates the cancer nature of the cells.

Procedure: Cells are plated in multi-well chamber slides fixed with 4% paraformaldehyde (10 min at room temperature), rinsed with PBS. Fixed cells were incubated with diluted primary antibodies (KRT18 - MAB12104 from Thermofisher, 1:300 and KRT19 – 14965-1AP from Thermofisher, 1:500; antibodies diluted with Antibody Dilution Buffer for ICC and IHC, SF40010) for 2 hours at room temperature, rinsed 3 times 15 min each in PBS pH7.4. Then incubated with fluorescent secondary antibodies (anti-mouse Cy2 and anti-rabbit Cy3 from Jackson ImmunoResearch) for 30 minutes at room temp., washed in PBS same as above and mounted using iBright medium (SF40000).

| Control Well 1 | Control Well 2 |
|---|---|
|  |  |



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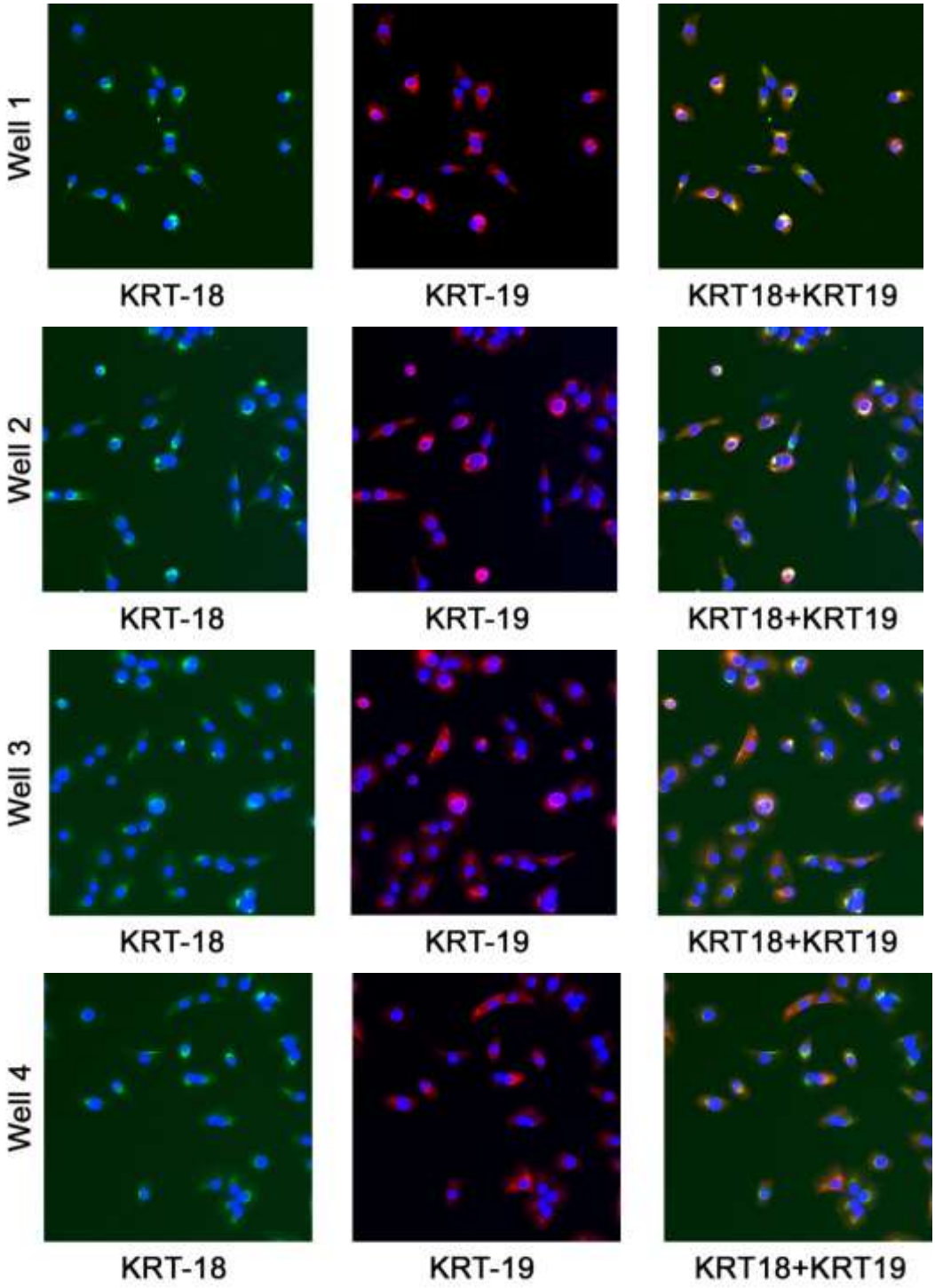


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