**Designation:** **NCI-H295R [H295R-S1]**

CLS order number: Cryovial: 300483
Vital: 330483

| Origin and General Characteristics | |
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| Organism: | Homo sapiens (human) |
| Ethnicity: | Black |
| Age: | 48 years old |
| Gender: | Female |
| Tissue: | Adrenal gland |
| Morphology: | Epithelial |
| Cell type: | Adrenocortical carcinoma |
| Growth Properties: | Monolayer, adherent |
| Description: | NCI-H295R was adapted from the NCI-H295 pluripotent adrenocortical carcinoma cell line established by A.F. Gazdar and associates (1990) from a carcinoma of the adrenal cortex. The original cells were adapted to a culture medium which decreased the population doubling time from 5 days to 2 days. The adapted cells were selected to grow in a monolayer, in contrast to the original cells which grew in suspension. This cell line retains the ability to produce adrenal androgens. It is responsive to angiotensin II and potassium ions.<br>According to the designation as described by Wang et al. (2012), which is based on the proliferation of the cells in differing culture media, the sub clone available at CLS is H295R-S1. |
| References: | Gazdar AF et al. Establishment and characterization of a human adrenocortical carcinoma cell line that expresses multiple pathways of steroid biosynthesis. Cancer Res 50: 5488-96, 1990.<br>Wang T, Rainey WE. Human Adrenocortical Carcinoma Cell Lines. Mol. Cell. Endocrinol. 351 (1): 58–65, 2012. PMC 3288152 Freely accessible. PMID 21924324. doi:10.1016/j.mce.2011.08.041. |
| **Culture Conditions and Handling** | |
| Culture Medium: | DMEM: Ham's F12 medium (1:1 mixture) containing 15 mM HEPES, 0.00625 mg/ml insulin, 0.00625 mg/ml transferrin, 6.25 ng/ml selenium, 1.25 mg/ml bovine serum albumin, and 0.00535 mg/ml linoleic acid and 2.5% Nu-Serum I. (MG-42, CLS order number 820402). |
| Subculturing: | Remove medium and rinse the adherent cells using PBS without calcium and magnesium (3-5 ml PBS for T25, 5-10ml for T75 cell culture flasks).<br>Add Accutase (1-2ml per T25, 2.5ml per T75 cell culture flask), the cell sheet must be covered completely.<br>Incubate at ambient temperature for 8-10 minutes.<br>Carefully resuspend the cells with medium (10 ml), centrifuge for 5 min at 300xg, resuspend cells in fresh medium and dispense into new flasks which contain fresh medium. |
| Split Ratio: | A ratio of 1:3 to 1:4 is recommended |
| Fluid Renewal: | 2 to 3 times weekly |
| Freeze Medium: | CM-ACF (CLS order number: 800650, 50ml) is a serum-free cryoprotective medium. |
| Freezing recovery: | Within 48 hrs past thawing |
| Sterility: | Fluorescence (DAPI) test: negative; Mycoplasma specific PCR: negative; Bacteria specific PCR: negative |
| Biosafety Level: | 1 |
| Safety precautions: | If the cryovial is planned to be stored in liquid nitrogen and to be thawed in the future, special safety precautions should be followed:<br>Protective gloves and clothing should be used and a facemask or safety goggles must be |

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| | worn when transferring frozen samples into or removing from the liquid nitrogen tank.<br>The removal of a cryovial from liquid nitrogen may result in the explosion of the frozen vial creating flying fragments.<br>Caputo, J.L. Biosafety procedures in cell culture. J. Tissue Cult. Methods 11:223-227, 1988. ATCC Quality Control Methods for Cell Lines, 2nd edition, 1992. |

| Special Features of the Cell Line | | |
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| Viruses: | SMRV: Negative, as confirmed by Real-Time PCR | |
| DNA Profile (STR): | Amelogenin: X,X<br>CSF1PO:10,12<br>D13S317: 13<br>D16S539: 11<br>D5S818: 12<br>D7S820: 9,12<br>TH01: 9.3<br>TPOX: 8 | vWA: 17,18<br>D3S1358: 15,16<br>D21S11: 32.2<br>D18S51: 17<br>Penta E: 5,12<br>Penta D: 8<br>D8S1179: 13<br>FGA: 19.2,24 |
| Products: | Aldosterone; cortisol; C19 steroids | |

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| Certificate of Analysis: | The Certificate of Analysis for each batch can be requested by e-mail at service@clsgmbh.de. |

| Recommendations for handling of adherent cell cultures following delivery | |
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| Cryopreserved cells | If immediate culturing is not intended, the cryovial(s) must be stored below -150°C or at least at -80°C after arrival.<br>If immediate culturing is intended, please follow these instructions:<br>Quickly thaw by rapid agitation in a 37°C water bath within 40-60 seconds. The water bath should have clean water containing an antimicrobial agent. As soon as the sample has thawed, remove the cryovial from the water bath. Note: A small ice clump should still remain and the vial should still be cold.<br>From now on, all operations should be carried out under aseptic conditions.<br>Transfer the cryovial to a sterile flow cabinet and wipe with 70% alcohol. Carefully open the vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of culture medium (room temperature). Resuspend the cells carefully. Centrifuge at 300xg for 3 min and discard the supernatant. The centrifugation step may be omitted, but in this case the remains of the freeze medium have to be removed 24 hours later.<br>Resuspend the cells carefully in 10ml fresh cell culture medium and transfer them into two T25 cell culture flasks. All further steps are described in the Subculture section. |
| Proliferating Cultures | The cell culture flasks are completely filled with cell culture medium to prevent loss of cells during transit. Remove the entire medium except for a sufficient volume to cover the floor of the flask. Incubate at 37°C for 24 hrs.<br>Sometimes the cultures are handled roughly during transit, and most of the cells detach and float in the culture medium. If this has occurred remove the entire content of the flask and centrifuge at 300x g for 5 minutes. Take off the supernatant, resuspend the cells in 10 ml of culture medium and transfer the entire cell suspension into cell culture flasks of suitable size (size (do not seed in more than 1T75 flask). |

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| Warranty: | CLS warrants for a high cell viability and culture performance only if the product(s) is (are) stored and cultured according to the information described above. Using cell culture media and supplements other than the ones recommended in this product information may result in satisfactory proliferation and viabilities. CLS, however, does not warrant for cell recovery, proliferation and function if differing formulations are employed. |
| Disclaimer: | The customer shall not be entitled to employ this product for purposes other than research. Commercial utilization shall not be permitted; in particular, the cell line, its components or materials made therefrom shall not be sold or transferred to any third party. In addition, the term ‘Commercial use’ shall mean any activity by a party for consideration and may include, but is not limited to, use of the product or its components in manufacturing, for providing services, e.g. fee for service testing, in quality control or |

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| | assurance processes within the manufacturing of products for sale, for therapeutic, diagnostic or prophylactic purposes, or for resale. |