ES-R1-EYFP YC5/EYFP  (Embryo, Mouse)

Culture Medium:
MEF medium consists of Advanced DMEM/F12, 10% FBS, 2 mM Glutamine and 0.1 mM β-mercaptoethanol.
KSR medium consists of KO-DMEM, 20% Knock-Out Serum Replacer, 2 mM Glutamine, NEAA, 0.1 mM β-mercaptoethanol and LIF 1000 Units/ml.

Cell Line Description:
Pluripotent mouse embryonic stem cell line expressing EYFP. This yellow fluorescent variant was generated by the random integration of EYFP transgenes into ES-R1 (EC07072001) using co-electroporation with a circular selectable marker containing vector pPGK Puro. The vector is driven by a CMV immediate early enhancer coupled to the chicken beta-actin promoter and first intron.

DESCRIPTION OF REPOSITORY REFERENCE SEED STOCK
Morphology: Adherent monolayer of spheroidal cells on feeder layer of mouse primary embryonic fibroblasts
Growth Mode: Adherent
Subculture Routine: Embryonic stem (ES) cells require the use of mitotically inactivated feeder cells to support the growth of stem cells in the undifferentiated state. Mouse embryonic fibroblasts, STO (EC86032003) or SNL 76/7 (EC07032801) can be used. At ECACC plastic ware is pre-coated with gelatine prior to plating feeder cells. Porcine gelatine is dissolved in sterile water (0.5g/500mL) at 56°C. The 0.1% solution is sterilized by filtration (0.22μm). Add 0.1% gelatine to plastic ware to cover bottom, and incubate for 20 minutes at room temperature. Remove gelatine, wash with PBS once and replace with appropriate culture medium. The flask/dish must not be allowed to dry out.
Feeder layers are prepared on the gelatinized flasks at least 24 hours in advance of being required. An ampoule is thawed in 37°C water bath and the contents quickly transferred to a 15ml centrifuge tube. MEF medium is added drop wise to 5mL. Cells are centrifuged at 150 x g for 5 minutes at Room Temperature (RT). Cells are resuspended in 5mL of MEF medium. Cells are counted and added to flasks containing the correct medium at 1-3 x 10^4 cells/cm².
An ampoule of ES cells is thawed in 37°C water bath and the contents quickly transferred to a 15mL centrifuge tube. KSR medium is added drop wise to 5mL. Cells are centrifuged at 150 x g for 5 minutes. Cells are resuspended in
5mL of KSR medium. The prepared feeder flask is washed once with PBS and KSR medium added. ES cells should be plated at 4-5 x 10^4 cells/cm². Cultures must be incubated in a humidified 5% CO₂/95% air incubator at 37°C. A 100% media change must be performed every day and cells passaged every 2-3 days. Colonies must not be allowed to touch each other as overgrowth will result in differentiation.

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**Originator:** Yes

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