CERTIFICATE OF ANALYSIS

Product Information

Product Name	HEK293/5-HT2C
Cat. No.	M00319
Lot No.	B80081804
Host Cell:	HEK293
Target Gene:	5-HT2C
Quantity:	2 vials of frozen cells, > $1x10^6$ cells/vial
Shipping Condition:	Dry Ice
Recommended Storage Condition:	Liquid Nitrogen

Stable Cell Line Information

Recommended Cell Culture Medium: DMEM+ 10% FBS + 300 μg/ml G418

Freeze Medium: 45% Culture Medium, 45%FBS, 10% (V/V) DMSO

Cell seeding Medium: DMEM+ 5% DFBS

Application: Functional assay for 5-HT2C

Test Item	Specification	Result
Lonza-180	Negative	Negative，Appendix 1
Hoechst Staining	Negative	Negative，Appendix 2
Functional assay	Calcium flux	EC_{50} = 0.23 nM

Appendix

Appendix 1: Lonza-180

Product Name	Ratio	Results	NC
HEK293/5-HT2C	0.86	Negative	0.18

Note: Positive Control: Ratio = Luminescence A/B >1; Negative Control: Ratio = Luminescence A/B<1; Luminescence A means read A (before the addition of the MycoAlert™ PLUS Substrate); Luminescence B means read B (after the addition of the MycoAlert™ PLUS Substrate);

Appendix 2: Hoechst Staining

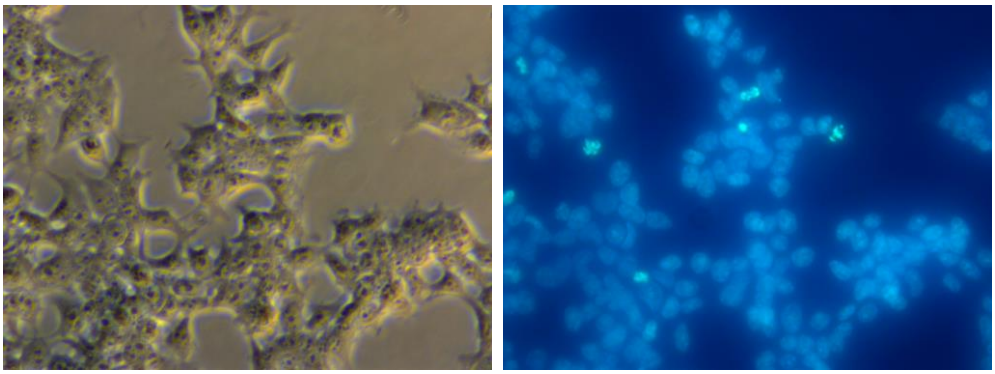

Figure 1. The cells were stained with Hoechst dye and observed under the microscope. No homogeneous little blue specks was detected and no mycoplasma contamination occurred

Appendix 3 : Calcium assay

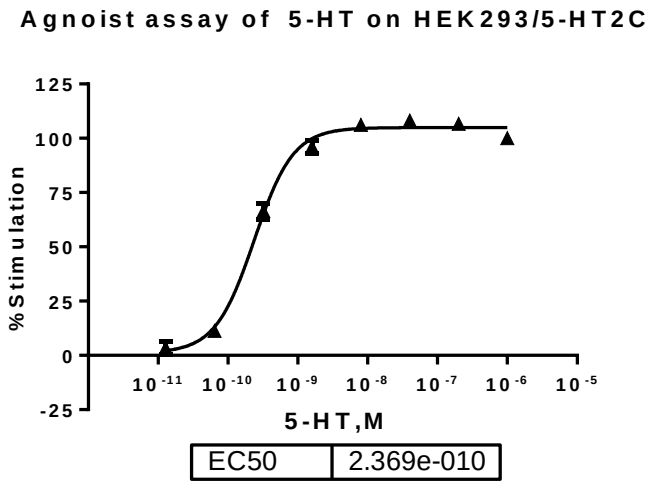

Figure 2. 5-HT-induced concentration-dependent stimulation of intracellular calcium mobilization in HEK293/5-HT2C cells. The cells were loaded with Calcium-4 prior to stimulation with a 5-HT2C receptor agonist, 5-HT. The intracellular calcium change was measured by FLIPR. The effects of agonist (%Stimulation) (Mean ± SD, n = 4) were plotted against the log of the cumulative doses (5-fold dilution) of 5-HT. The EC50 of 5-HT on 5-HT2C in HEK293 cells was 0.23 nM. The S/B of 5-HT on 5-HT2C in HEK293 cells was 5.3.

Caution

For research use only. Not intended for household use. If you have any questions about the Certificate of Analysis, please contact our customer service representative at 1-877-436-7274 (Toll-Free), or 1-732-885-9188.

Certified by: Leon Soy Date: 05/16/2018

Department of Biologics Development Director