

## Catalogue of validated fluorescent probes

In the following tables we list the available ligands, for **GPCRs**, **E3 Ligases** and **Intracellular Receptors** with information about their selectivity, the emission and excitation wavelengths, their affinity measured by a radioligand binding assay and the specific further assays in which they have been validated. We also include two fluorescent **GPTyS** for GPCR functional assays.

### GPCR fluorescent ligands

Receptor	Code	Cat number	$\lambda_{exc}/\lambda_{em}$	Affinity <sup>a</sup>	Selectivity <sup>a</sup>	Validation
<b>Dopamine Receptor</b>						
D <sub>2</sub>	CELT-074	DR-589-3	589/616	1.1 nM	<b>Selective</b> K <sub>i</sub> (D <sub>3</sub> ) = 136.5 nM K <sub>i</sub> (D <sub>4</sub> ) = 152.7 nM	Fluorescence Microscopy in transfected cells Flow cytometry
	CELT-426	DR-560-1	560/571	89.3 nM	<b>Partially Selective</b> K <sub>i</sub> (D <sub>3</sub> ) = 194.8 nM K <sub>i</sub> (D <sub>4</sub> ) = 263.0 nM	Fluorescence polarization Flow cytometry
	CELT-075	DR-743-1	748/776	3.2 nM	<b>Selective</b> K <sub>i</sub> (D <sub>3</sub> ) = 294.6 nM K <sub>i</sub> (D <sub>4</sub> ) = 220.3 nM	Fluorescence Microscopy in transfected cells (ongoing)
D <sub>3</sub>	CELT-429	DR-589-7	589/616	75.4 nM	<b>Selective</b> (% displ. 1 $\mu$ M) D <sub>2</sub> = 6% D <sub>4</sub> = 3%	Fluorescence Microscopy in transfected cells (ongoing)
	CELT-419	DR-560-2	560/571	65.6 nM	<b>Partially Selective</b> K <sub>i</sub> (D <sub>2</sub> ) = 151.4 nM	Fluorescence polarization
D <sub>2</sub> /D <sub>3</sub>	CELT-240	DR-589-6	589/616	D <sub>3</sub> = 2.1 nM D <sub>2</sub> = 2.3 nM	<b>Selective against D<sub>4</sub></b> (% displ. 1 $\mu$ M) D <sub>4</sub> = 1%	Flow cytometry
	CELT-241	DR-646-1	646/662	D <sub>3</sub> = 4.8 nM D <sub>2</sub> = 5.2 nM	<b>Selective against D<sub>4</sub></b> K <sub>i</sub> (D <sub>4</sub> ) = 302.5 nM	Fluorescence Microscopy in transfected cells (ongoing)
<b>Adenosine Receptor</b>						
PAN-ADO	CELT-298	ADOR-646-1	646/662	A <sub>1</sub> = 20.9 nM A <sub>2A</sub> = 171.0 nM A <sub>2B</sub> = 44.7 nM A <sub>3</sub> = 95.2 nM	<b>Non Selective</b>	Fluorescence Microscopy in transfected cells
A <sub>1</sub>	CELT-448	ADOR-560-1	560/571	26.2 nM	<b>Selective</b> (% displ. 1 $\mu$ M) A <sub>2A</sub> = 11% A <sub>2B</sub> = 22% A <sub>3</sub> = 24%	Fluorescence polarization (ongoing) Fluorescence Microscopy in transfected cells (ongoing)

	<b>CELT-372</b> (A <sub>1</sub> /A <sub>2B</sub> )	ADOR-589-1	589/616	A <sub>1</sub> = 1.9 nM A <sub>2B</sub> = 24.7 nM	<b>Partially Selective</b> K <sub>i</sub> (A <sub>2A</sub> ) = 80.3 nM K <sub>i</sub> (A <sub>3</sub> ) = 967.8 nM	Fluorescence Microscopy in transfected cells
	<b>CELT-360</b>	ADOR-646-2	646/662	8.6 nM	<b>Non Selective</b> K <sub>i</sub> (A <sub>2A</sub> ) = 98.4 nM K <sub>i</sub> (A <sub>2B</sub> ) = 72.2 nM K <sub>i</sub> (A <sub>3</sub> ) = 231.0 nM	Fluorescence Microscopy in transfected cells
<b>A<sub>2A</sub></b>	<b>CELT-316</b>	ADOR-589-2	589/616	116.1 nM	<b>Selective</b> (% displ. 1 μM) A <sub>1</sub> = 18 % A <sub>2B</sub> = 33 % A <sub>3</sub> = 31 %	Fluorescence Microscopy in native cells
	<b>CELT-300</b>	ADOR-646-4	646/662	8.4 nM	<b>Selective</b> (% displ. 1 μM) A <sub>1</sub> = 31 % A <sub>2B</sub> = 18 % A <sub>3</sub> = 38 %	Fluorescence Microscopy in transfected cells (ongoing)
<b>A<sub>2B</sub>/A<sub>3</sub></b>	<b>CELT-327</b>	ADOR-589-4	589/616	A <sub>2B</sub> = 35.6 nM A <sub>3</sub> = 45.7 nM	<b>Selective</b> (% displ. 1 μM) A <sub>1</sub> = 41 % A <sub>2A</sub> = 1 %	Fluorescence Microscopy in native cells <sup>1</sup>
<b>A<sub>3</sub></b>	<b>CELT-228</b>	ADOR-560-2	560/571	52.7 nM	<b>Selective</b> (% displ. 1 μM) A <sub>1</sub> = 2 % A <sub>2A</sub> = 1 % A <sub>2B</sub> = 5 %	Fluorescence Microscopy in native cells; Fluorescence polarization <sup>1,2</sup>
	<b>CELT-071</b>	ADOR-589-7	589/616	6.1 nM	<b>Selective</b> (% displ. 1 μM) A <sub>1</sub> = 2 % A <sub>2A</sub> = 2 % A <sub>2B</sub> = 2 %	Fluorescence Microscopy in transfected and native cells
	<b>CELT-480</b>	ADOR-646-6	646/662	12.0 nM	<b>Selective</b> (% displ. 1 μM) A <sub>1</sub> = 38 % A <sub>2A</sub> = 23 % A <sub>2B</sub> = 49 %	Fluorescence Microscopy in transfected cells (ongoing)
<b>Serotonin Receptor</b>						
<b>PAN-5-HT<sub>2</sub></b>	<b>CELT-386</b>	DR-589-2	589/616	5-HT <sub>2A</sub> = 4.9 nM 5-HT <sub>2B</sub> = 53.0 nM 5-HT <sub>2C</sub> = 47.8 nM	<b>Non Selective</b>	Fluorescence Microscopy in transfected cells (ongoing)
<b>5-HT<sub>2A</sub></b>	<b>CELT-426</b>	DR-560-1	560/571	6.2 nM	<b>Selective</b> (% displ. 1 μM) 5-HT <sub>2B</sub> = 12 % 5-HT <sub>2C</sub> = 35 %	Fluorescence Microscopy in transfected cells (ongoing)

<b>5-HT<sub>2B</sub></b>	<b>CELT-211</b>	5HT-589-1	589/616	56.3 nM	<b>Selective</b> (% displ. 1 $\mu$ M) 5-HT <sub>2A</sub> = 0.9 % 5-HT <sub>2C</sub> = 1.7 %	Fluorescence Microscopy in transfected cells
<b>Cannabinoid Receptor</b>						
<b>PAN-CB</b>	<b>CELT-335</b>	CBR-646-1	646/662	CB <sub>1</sub> = 44.8 nM CB <sub>2</sub> = 7.4 nM	<b>Non Selective</b>	HTRF in adherent cells <sup>3,4</sup> High Content screening Fluorescence Microscopy in transfected cells
<b>CB<sub>2</sub></b>	<b>CELT-331</b>	CBR-646-3	646/662	75.9 nM	<b>Selective</b> (% displ. 1 $\mu$ M) CB <sub>1</sub> = 20 %	High Content screening Fluorescence Microscopy in transfected cells
<b>Muscarinic Receptor</b>						
<b>M<sub>1</sub>/M<sub>2</sub></b>	<b>CELT-249</b>	MUSCR-589-2	589/616	M <sub>1</sub> = 133.0 nM M <sub>2</sub> = 11.5 nM	No data	Fluorescence Microscopy in transfected cells (ongoing)
	<b>CELT-095</b>	MUSCR-743-1	748/776	M <sub>1</sub> = 57.8 nM M <sub>2</sub> = 37.7 nM	No data	Fluorescence Microscopy in transfected cells (ongoing)
<b>Adrenergic Receptor</b>						
<b><math>\alpha</math><sub>1A</sub></b>	<b>CELT-033</b>	ADR-560-1	560/571	5.0 nM	<b>Selective</b> (% displ. 1 $\mu$ M) $\alpha$ <sub>2A</sub> = 15 %	Fluorescence Microscopy in transfected cells (ongoing)
	<b>CELT-030</b>	ADR-646-1	646/662	28.3 nM	<b>Selective</b> K <sub>i</sub> ( $\alpha$ <sub>2A</sub> ) = 1081 nM	Fluorescence Microscopy in transfected cells (ongoing)
<b>C5a Receptor</b>						
<b>C5aR</b>	<b>CELT-058</b>	C5aR-646-1	646/662	24.9 nM	Not Defined	Flow cytometry
<b>Angiotensin Receptor</b>						

<b>AT<sub>1</sub></b>	<b>CELT-045</b>	ATR-646-1	646/662	160.0 nM	<b>Selective</b> (% displ. 1 $\mu$ M) AT <sub>2</sub> = 8.4 %	Fluorescence Microscopy in transfected cells (ongoing)
	<b>CELT-252</b>	ATR-560-1	560/571	39.0 nM	<b>Selective</b> (% displ. 1 $\mu$ M) AT <sub>2</sub> = 3.3 %	Fluorescence Microscopy in transfected cells (ongoing)
<b>GLP1 Receptor</b>						
<b>GLP1</b>	<b>CELT-111</b> (LUXendin551)	GLP1-551-1	551/576	7.2	<b>Selective</b> <sup>8</sup>	Widefield/confocal/2-photon microscopy in live and fixed mammalian cells and tissue, as well as anaesthetized mice <sup>5</sup>
	<b>CELT-112</b> (LUXendin645)	GLP1-645-1	645/664	7.5	<b>Selective</b> <sup>9</sup>	Widefield/confocal/2-photon microscopy in live and fixed mammalian cells and tissue, as well as anaesthetized mice <sup>6</sup> TR-FRET
	<b>CELT-113</b> (LUXendin762)	GLP1-762-1	762/784	7.0	<b>Selective</b> <sup>9</sup>	Widefield/confocal/2-photon microscopy in live and fixed mammalian cells and tissue, as well as anaesthetized mice. <sup>8</sup> Non-invasive fluorescence preclinical imaging <sup>7-9</sup>
<b>Oxytocin Receptor</b>						
<b>OTR</b>	<b>CELT-114</b>	OTR-597-1	597/657 (water)	0.54 nM	Not Defined	TR-FRET and confocal microscopy <sup>10</sup>
	<b>CELT-115</b>	OTR-650-1	650/667	1.59 nM	<b>Selective</b> >1000 nM (V1aR and V1bR), 509 nM (V2R)	TR-FRET and confocal microscopy <sup>11</sup>

<sup>a</sup>K<sub>i</sub> or % of displacement at 1  $\mu$ M measured by radioligand binding assay. In the case of the C5aR ligand corresponds to the EC<sub>50</sub>. In the case of GLP1R ligands correspond to pIC<sub>50</sub>. In the case of OTR ligands, K<sub>i</sub> was determined by competition experiments against [<sup>3</sup>H]AVP (labelled arginine vasopressin) for CELT-114 and K<sub>d</sub> by TR-FRET saturation binding experiments for CELT-115.

## Probes for GPCRs functional assays

target	Code	Cat number	$\lambda_{exc}/\lambda_{em}$	Description	Validation
G protein	<b>CELT-505</b>	G-FUN-503-1	503/506	<b>fluorescent GTP<math>\gamma</math>S-green</b>	G protein activation assays in steady state and kinetic mode
	<b>CELT-503</b>	G-FUN-589-1	589/616	<b>fluorescent GTP<math>\gamma</math>S-red</b>	G protein activation assays in steady state and kinetic mode

## E3 ligases fluorescent ligands

E3 ligase	Code	Cat number	$\lambda_{exc}/\lambda_{em}$	Affinity <sup>a</sup>	Selectivity	Validation
VHL	<b>CELT-504</b>	VHL-564-1	564/570	2.3 nM	Not defined	-
	<b>CELT-050</b>	VHL-646-1	646/662	96.0 nM	Not defined	TR-FRET competition binding
CRBN	<b>CELT-077</b>	CRBN-589-1	589/616	20.0 nM	Not defined	-
	<b>CELT-081</b>	CRBN-646-1	646/662	14.0 nM	Not defined	-

<sup>a</sup>  $K_d$  in fluorescence polarization competition binding.

## Intracellular receptors fluorescent ligands

Receptor	Code	Cat number	$\lambda_{exc}/\lambda_{em}$	Affinity <sup>a</sup>	Selectivity <sup>a</sup>	Validation
<b>SIGMA receptor</b> $\sigma_1 / \sigma_2$	<b>CELT-483</b>	$\sigma$ R-646-1	646/662	$\sigma_1 = 51.3$ nM $\sigma_2 = 30.2$ nM	Non Selective	Flow cytometry <sup>12</sup> Confocal microscopy <sup>12</sup> Live cell microscopy <sup>12</sup>
	<b>CELT-483 + Masking agents<sup>b</sup></b>	$\sigma$ R-BOX-1				

<sup>a</sup>  $K_i$  by radioligand binding assay.

<sup>b</sup> We also provide kits of CELT-483 together with potent and selective  $\sigma_1$  and  $\sigma_2$  receptors masking agents (L6 and F390, respectively).

# Catalogue of validated **biotinylated ligands**

In the following tables we list the available ligands for **GPCRs Receptors** with information about their selectivity and their affinity measured by a radioligand binding assay.

## GPCR ligands

Receptor	Code	Cat number	Affinity <sup>a</sup>	Selectivity <sup>a</sup>
<b>Cannabinoid Receptor</b>				
<b>CB<sub>2</sub></b>	<b>CELT-506</b>	CB2-BTN-1	41.8 nM	<b>Selective</b> (% displ. 1 $\mu$ M) CB <sub>1</sub> = 25 %

<sup>a</sup> K<sub>i</sub> by radioligand binding assay.

In case you are interested by any of these ligands and they have not been validated yet for the kind of assay you want to test, please let us know: based on our experience we could advise you about which fluorophore works better for each assay, and eventually develop new versions -i.e. keeping the pharmacophore but changing the fluorophore- in a turnaround of 4-6 weeks.

### **Contact Information**

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