B'SYS GmbH

$5HT_3A$ and $5HT_3A/B$ Assay

Specification Sheet

TABLE OF CONTENTS

1 BACKGROUND

1.1 Human 5-hydroxytryptamine (Serotonin) Receptors

Serotonin receptors belong to the ligand-gated ion channel receptor superfamily. These receptors are activated by 5-hydroxytryptamine (serotonin) which is a biogenic hormone that functions as a neurotransmitte and a mitogen. This receptor causes fast, depolarizing responses in neurons after activation

1.2 B'SYS' 5HT$_3$A / 5HT$_3$A/B Assays

B'SYS has designed an assay on a HEK-293 cell line with constitutive expression of either human 5HT3A or coexpression of human 5HT$_3$A/B receptors as well as a CHO cell line expressing the human 5HT3A receptor. The human 5HT$_3$A or 5HT$_3$A/B cDNA were cloned and transfected into HEK-293 cells and then the functional properties of the human 5HT$_3$A or 5HT$_3$A /B receptors were validated by means of the patch-clamp technique.

2 VALIDATION OF HEK-293 5HT$_3$A / 5HT$_3$A/B ASSAY

2.1 Agonist Screen

5HT$_3$A were measured by means of the patch-clamp technique in the whole-cell configuration. The bath solution contained (in mM) NaCl 137, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 10 and Glucose 10. The pH was adjusted to 7.4 with NaOH. The pipette solution consisted of (in mM) KCl 130, $MgCl_2$ 1, MgATP 5, HEPES 10, EGTA 5. The pH was adjusted to 7.2 with KOH. After formation of a Gigaohm seal between the patch electrodes and individual 5HT$_3$A or 5HT$_3$A/B stably transfected HEK-293 cells, the cell membrane across the pipette tip was ruptured to assure electrical access to the cell interior. All solutions applied to cells were continuously perfused and maintained at room temperature. As soon as a stable seal could be established, a holding potential of -80 mV was applied. Test solutions were applied by a fast multichannel application system positioned in the vicinity of a cell. 5HT$_3$A and 5HT$_3$A/B channels were activated for 3 s in intervals of at least 45 s with manual patch-clamp and activated for 4 s in intervals of at least 45 s with automated patch-clamp. Test items were applied at increasing concentrations.

As example for the agonistic screen the concentration dependence of serotonin on 5HT$_3$A was investigated manually and on the QPatch. For 5HT$_3$A/B, the concentration dependence of serotonin was investigated manually. The results were as follows:

	EC_{50}	**Hill coefficient**
5HT$_3$A manual	6.56 µM	1.16
5HT$_3$A automated	4.27 µM	1.46
5HT$_3$A/B manual	6.16 µM	1.36

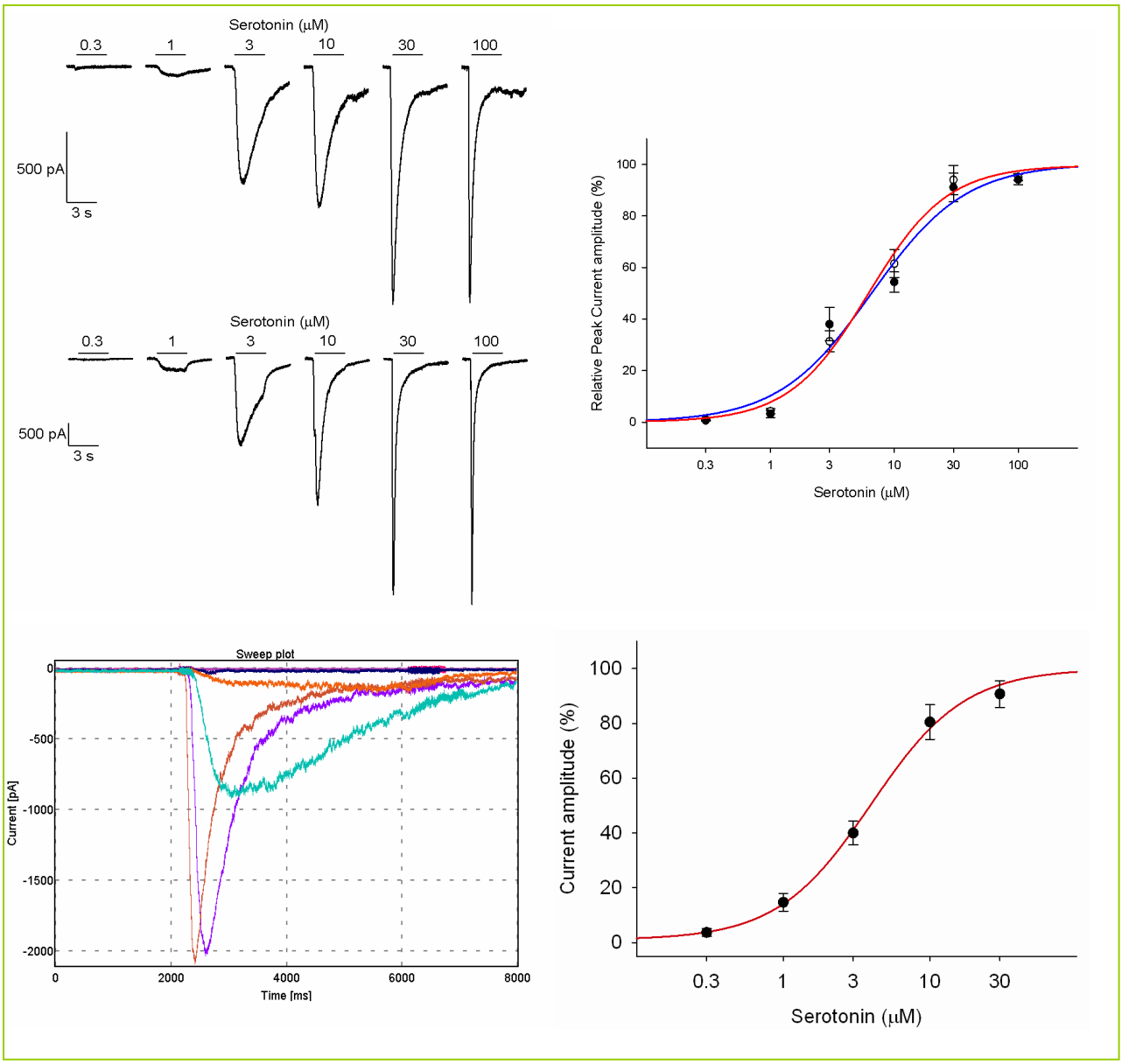

Figure 1: Upper traces: Representative current recordings from manually measured 5HT$_3$A and 5HT$_3$A /B HEK-293 cells stimulated with 0.3, 1.0, 3.0, 10, 30 and 100 µM serotonin (upper trace: 5HT$_3$A; lower trace: 5HT$_3$A/B), dose response curve for serotonin, blue fit: 5HT$_3$A with EC$_{50}$: 6.56 µM, red fit: 5HT$_3$A/B with EC$_{50}$: 6.16 µM with a Hill coefficient of 1.16..
Lower trace: Representative current recordings from 5HT$_3$A HEK-293 cells measured on the QPatch stimulated with 0.3, 1.0, 3.0, 10 and 30 µM serotonin; dose response curve for serotonin, EC$_{50}$: 4.27 µM with a Hill coefficient of 1.46.

2.2 Antagonist Screen

5HT$_3$A or 5HT$_3$A/B currents were measured by means of the patch-clamp technique in the whole-cell configuration. The bath solution contained (in mM) NaCl 137, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 10 and Glucose 10. The pH was adjusted to 7.4 The pipette solution consisted of (in mM) KCl 130, $MgCl_2$ 1, MgATP 5, HEPES 10, EGTA 5. The pH was adjusted to 7.2 with KOH. After formation of a Gigaohm seal between the patch electrodes and individual 5HT$_3$A or 5HT$_3$A/B stably transfected HEK-293 cells, the cell membrane across the pipette tip was ruptured to assure electrical access to the cell interior. All solutions applied to cells were continuously perfused and maintained at room temperature. As soon as a stable seal could be established, an holding potential of -80 mV was applied. Test solutions were applied by a fast multichannel application system positioned in the vicinity of a cell. 5HT$_3$A or 5HT$_3$A/B channels were activated for 3 s in intervals of at least 1 min. Test items were applied at increasing concentrations.

As examples for the antagonistic screen, the concentration dependence of Tubocurarine and Palonosetron were investigated in the presence of 10 µM serotonin for manual patch-clamp and the concentration dependence of Palonosetron was investigated in presence of 3 µM serotonin for automated patch-clamp.

The representative current traces for 5HT$_3$A and 5HT$_3$A/B cells are given below:

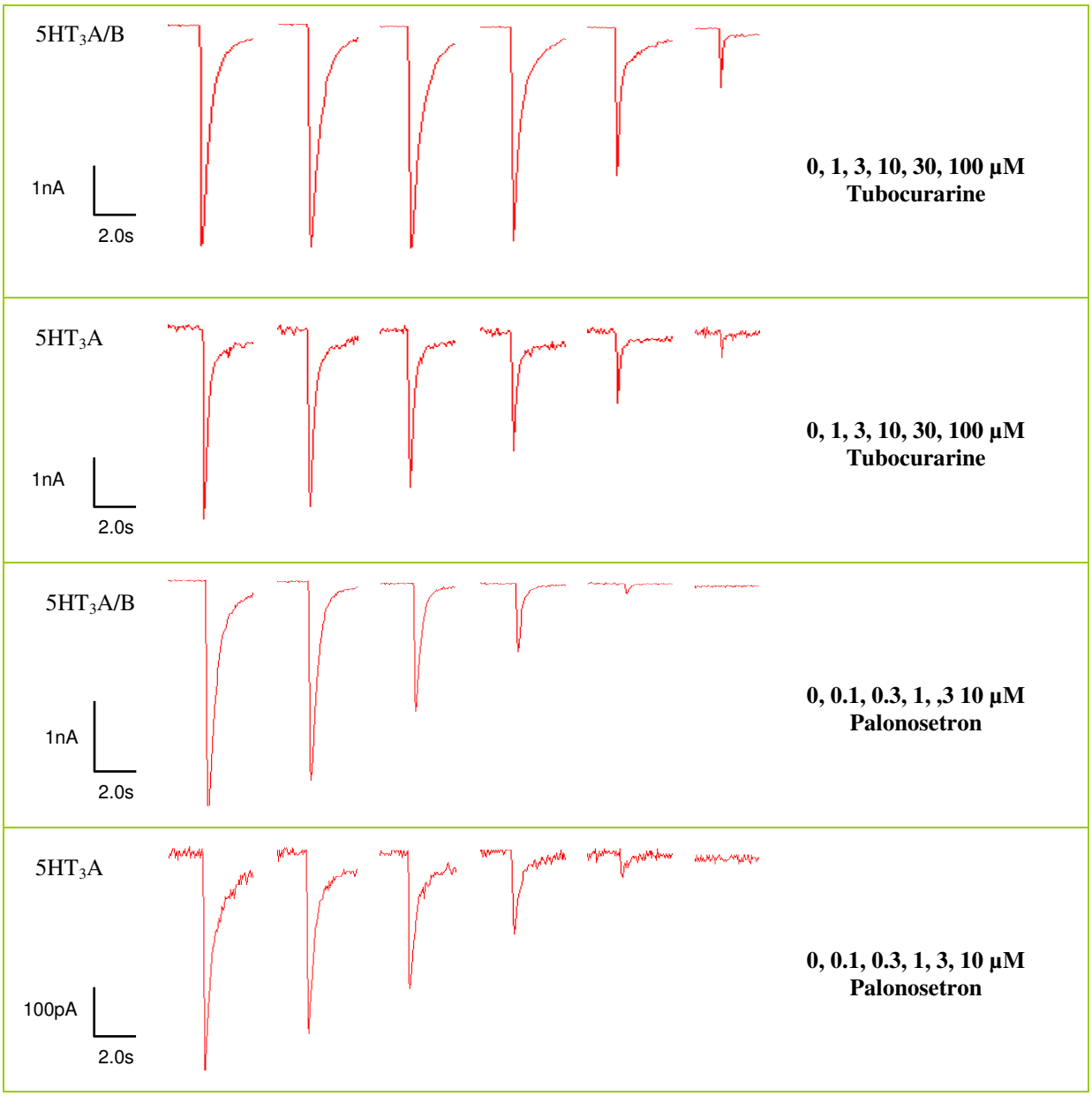

Figure 2: Representative current traces for 5HT$_3$A or 5HT$_3$A/B receptors treated with Tubocurarine or Palonosetron

The IC_{50} values were determined:

Receptor Type	Tubocurarine IC_{50}	Palonosetron IC_{50}
5HT$_3$A manual	12.02 µM	590.2 nM
5HT$_3$A automated	-	35.1 nM
5HT$_3$A/B manual	55.85 µM	449.8 nM

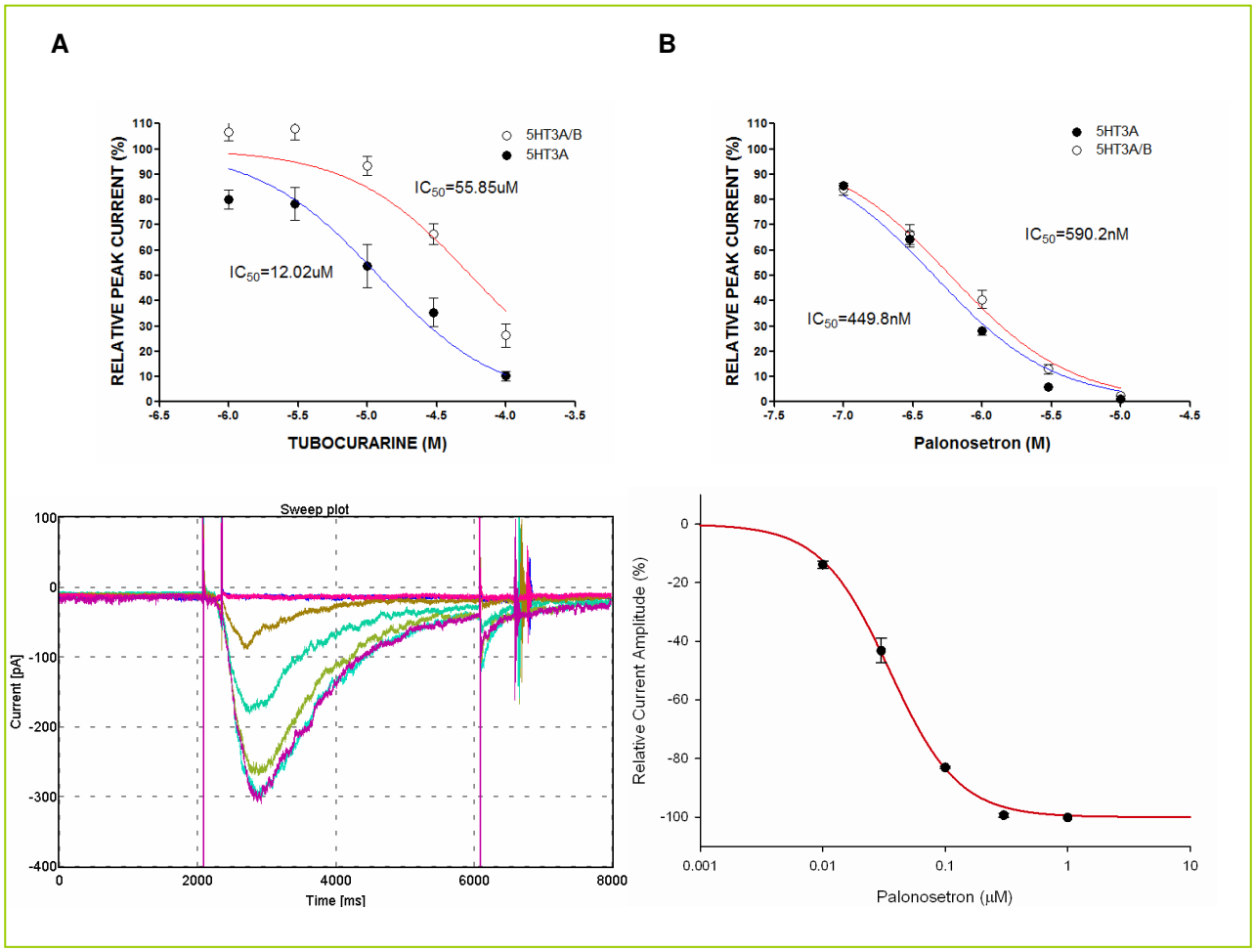

Figure 3: upper traces: dose response curves for 5HT$_3$A or 5HT$_3$A/B receptors measured manually a) Tubocurarine, b) Palonosetron; lower traces: representative traces and dose response curve for 5HT$_3$A on the QPatch for Palonosetron.

3 VALIDATION OF CHO 5HT3A ASSAY

3.1 Agonist Screen

5HT$_3$A were measured by means of the patch-clamp technique in the whole-cell configuration. The bath solution contained (in mM) NaCl 137, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 10 and Glucose 10. The pH was adjusted to 7.4 with NaOH. The pipette solution consisted of (in mM) KCl 130, $MgCl_2$ 1, MgATP 5, HEPES 10, EGTA 5. The pH was adjusted to 7.2 with KOH. After formation of a Gigaohm seal between the patch electrodes and individual 5HT$_3$A stably transfected CHO cells, the cell membrane across the pipette tip was ruptured to assure electrical access to the cell interior. All solutions applied to cells were continuously perfused and maintained at room temperature. As soon as a stable seal could be established, a holding potential of -80 mV was applied. Test solutions were applied by a fast multichannel application system positioned in the vicinity of a cell. 5HT$_3$A channels were activated for 4 s in intervals of at least 45 s with automated patch-clamp. Test items were applied at increasing concentrations.

As example for the agonistic screen the concentration dependence of serotonin on 5HT$_3$A was investigated on the QPatch. The results were as follows:

	EC_{50}	**Hill coefficient**
CHO 5HT$_3$A automated	3.53 µM	1.92

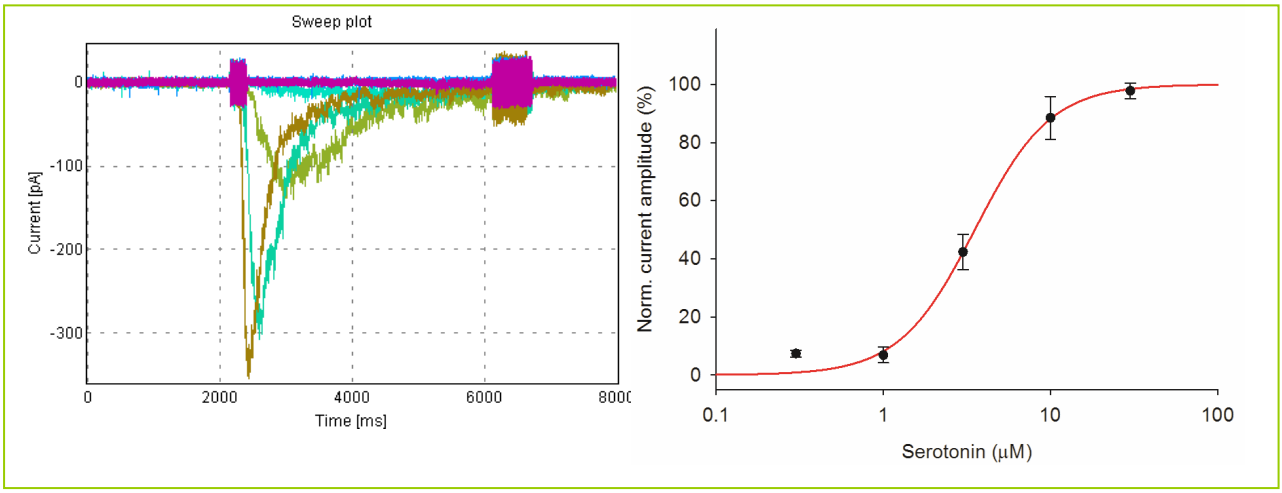

Figure 4: representative current trace and dose response curve for CHO 5HT$_3$A on the QPatch for Serotonin with a EC_{50} of 3.53 µM (Hill coefficient: 1.92)

3.2 Antagonist Screen

5HT$_3$A currents were measured by means of the patch-clamp technique in the whole-cell configuration. The bath solution contained (in mM) NaCl 137, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 10 and Glucose 10. The pH was adjusted to 7.4 The pipette solution consisted of (in mM) KCl 130, $MgCl_2$ 1, MgATP 5, HEPES 10, EGTA 5. The pH was adjusted to 7.2 with KOH. After formation of a Gigaohm seal between the patch electrodes and individual 5HT$_3$A stably transfected CHO cells, the cell membrane across the pipette tip was ruptured to assure electrical access to the cell interior. All solutions applied to cells were continuously perfused and maintained at room temperature. As soon as a stable seal could be established, an holding potential of -80 mV was applied. Test solutions were applied by a fast multichannel application system positioned in the vicinity of a cell. 5HT$_3$A channels were activated for 4 s in intervals of at least 45 s with automated patch-clamp. Test items were applied at increasing concentrations.

As examples for the antagonistic screen, the concentration dependence of Palonosetron was investigated in the presence of 3 µM serotonin for automated patch-clamp.

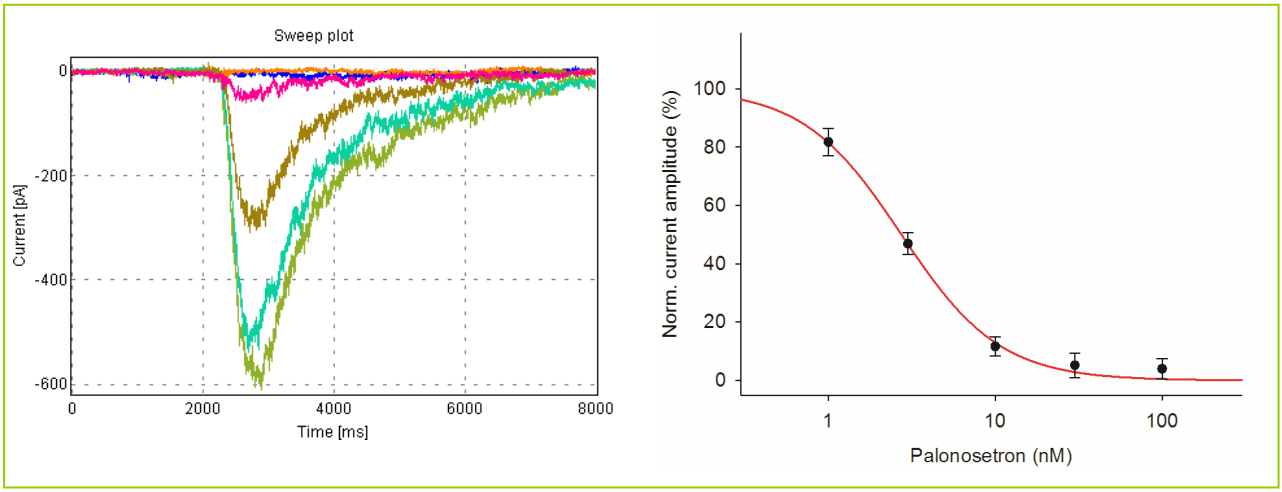

Figure 5: representative current trace and dose response curve for CHO 5HT$_3$A on the QPatch for Palonosetron with a IC_{50} of 2.74 nM (Hill coefficient: 1.47)

4 CONTACT INFORMATION

4.1 Contact Address

- B'SYS GmbH
  Technology Center Witterswil
  Benkenstrasse 254
  4108 Witterswil
  Switzerland

  Tel: +41 61 721 77 44
  Fax: +41 61 721 77 41
  Email: info@bsys.ch
  Web: www.bsys.ch