# $GABA_A$ Receptors Application Note

$GABA_A$ receptors represent a large and complex family of ion channels that consist of at least 16 subunits ($\alpha_{1-6}$, $\beta_{1-3}$, $\gamma_{1-3}$, $\delta$, $\varepsilon$, $\pi$, $\theta$) and have been shown to be vital for the normal functioning of the brain. These ligand-gated chloride ion channels form pentameric structures and are activated by γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the CNS, resulting in an inhibitory effect on neurotransmission.

$GABA_A$ receptors have been shown to be important in sedation, muscle relaxation and anaesthesia as well as being key drug targets for the treatment of neuronal disorders such as epilepsy and anxiety.

Here we show validation of fluorescence and automated electrophysiology assays designed to assess agonists, antagonists and allosteric modulators of these receptors, culminating in a high-throughput electrophysiology assay suited to assessing multiple $GABA_A$ receptor subtypes on a single assay plate.

**Generation of cell lines**

A panel of 19 inducible HEK cell lines over-expressing combinations of human $GABA_A$ subunits was generated (Table 1) by stable transfection and expansion of clonal isolates. Stable clones were verified for subunit expression by western blot and sequence analysis followed by functional assessment using a fluorescence-based FLIPR assay.

| $GABA_A$ receptor cell lines | | | |
|---|---|---|---|
| $\alpha_1\beta_1\gamma_2$ | $\alpha_1\beta_2\gamma_2$ | $\alpha1\beta_3\gamma_2$ | $\alpha_4\beta_3\delta$ |
| $\alpha_2\beta_1\gamma_2$ | $\alpha_2\beta_2\gamma_2$ | $\alpha_2\beta_3\gamma_2$ | |
| $\alpha_3\beta_1\gamma_2$ | $\alpha_3\beta_2\gamma_2$ | $\alpha_3\beta_3\gamma_2$ | |
| $\alpha_4\beta_1\gamma_2$ | $\alpha_4\beta_2\gamma_2$ | $\alpha_4\beta_3\gamma_2$ | |
| $\alpha_5\beta_1\gamma_2$ | $\alpha_5\beta_2\gamma_2$ | $\alpha_5\beta_3\gamma_2$ | |
| $\alpha_6\beta_1\gamma_2$ | $\alpha_6\beta_2\gamma_2$ | $\alpha_6\beta_3\gamma_2$ | |

Table 1: Panel of recombinant $GABA_A$ stable cell lines.

In the first instance, cell lines were functionally validated using a fluorescence-based FLIPR assay in which application of increasing concentrations of agonist (GABA) resulted in a concentration-dependent increase in response. Subsequently, dose-dependent block of $EC_{50}$ -activated $GABA_A$ responses was shown in the presence of increasing concentrations of the reference inhibitor Picrotoxin. Figure 1 shows Picrotoxin concentration-response curves for $\alpha_1$ - $\alpha_6$ ($\beta_1\gamma_2$). Example GABA $EC_{50}$ and Picrotoxin $IC_{50}$ values for $\beta_1$ $GABA_A$ subtypes are displayed in Table 2.

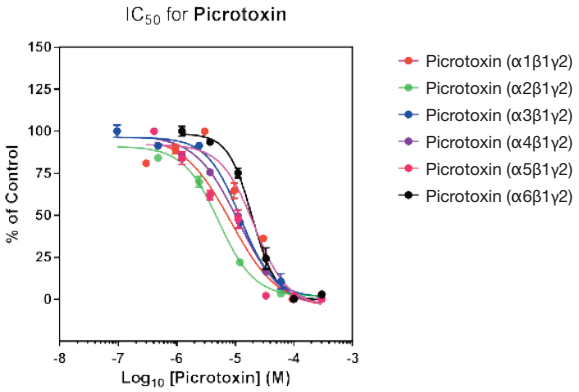


Figure 1: Effect of increasing Picrotoxin concentration on activated $GABA_A$ $\alpha_1$-$\alpha_6$ ($\beta_1\gamma_2$) receptors.

| Subtype | $EC_{50}$ | $IC_{50}$ |
|---|---|---|
| $\alpha_1\beta_1\gamma_2$ | 2.9 µM | 20 µM |
| $\alpha_2\beta_1\gamma_2$ | 2.9 µM | 5.2 µM |
| $\alpha_3\beta_1\gamma_2$ | 5.3 µM | 12 µM |
| $\alpha_4\beta_1\gamma_2$ | 1.0 µM | 11 µM |
| $\alpha_5\beta_1\gamma_2$ | 3.5 µM | 8.3 µM |
| $\alpha_6\beta_1\gamma_2$ | 0.5 µM | 19 µM |

Table 2: Comparison of $EC_{50}$ (GABA) and $IC_{50}$ (Picrotoxin) values across $\alpha_1$-$\alpha_6$ ($\beta_1\gamma_2$) subtypes.

**Electrophysiology**

Electrophysiology studies were performed using the SyncroPatch 384PE automated electrophysiology platform (Nanion Technologies GmbH). A stacked addition protocol was used to rapidly apply and remove GABA to enable reproducible activation of the receptor.

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**Agonist mode**

Test compound was applied to cells followed by application of a high concentration of GABA to confirm presence of functional $GABA_A$ receptors. A wash step was included between applications to ensure removal of residual compound. An example current trace is shown in Figure 2 in which control test compound (GABA $EC_{50}$) was applied followed by maximum GABA concentration.

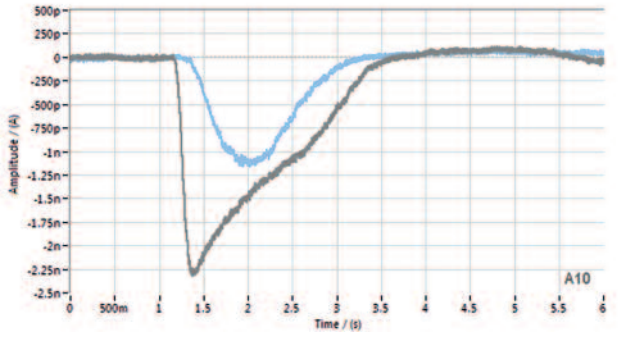


Figure 2. $GABA_A$ agonist assay showing currents induced by GABA $EC_{50}$ (blue) and maximum concentration of GABA (grey).

Reproducibility was assessed over multiple experiments on separate days with GABA $EC_{50}$ values remaining consistent between experiments (Figure 3).

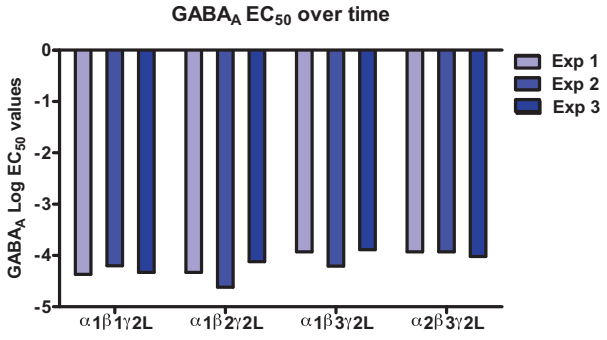


Figure 3. Analysis of $EC_{50}$ consistency over time.

**Antagonist mode**

To assess antagonists, GABA was applied, washed and re-applied in order to show reproducibility of signal prior to pre-incubation of compound and subsequent application of compound in the presence of GABA. Following a further wash step, GABA was re-applied to confirm reversibility of compound and finally a high concentration of reference blocker was used to verify full block (Figure 4).

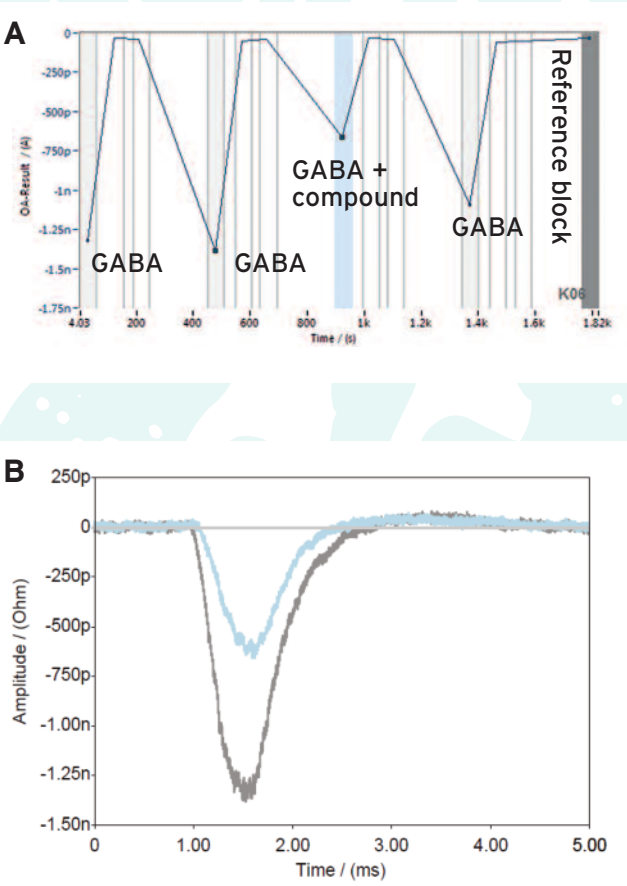


Figure 4: A) Time-course showing effect of Bicuculline on $GABA_A$ currents. B) Corresponding current trace showing GABA alone (grey) and GABA plus Bicuculline $IC_{50}$ (blue).

**Allosteric modulator mode**

To assess positive allosteric modulators, two different application methods were developed. In the first method, similar to the antagonist application protocol, GABA ($EC_{10}$) was applied twice followed by pre-incubation of compound and then GABA in the presence of the compound, as illustrated in the time-course in Figure 5A. In the second method, there was no pre-incubation of the modulator and instead GABA ($EC_{10}$) was applied to the cells, directly followed by GABA in the presence of the modulator, as shown in the current trace in Figure 5B. As expected, the control PAM Diazepam displayed modulator activity with GABA cell lines containing the $\alpha_1$, $\alpha_2$, $\alpha_3$ and $\alpha_5$ but not the $\alpha_4$ and $\alpha_6$ subunits (Figure 6).

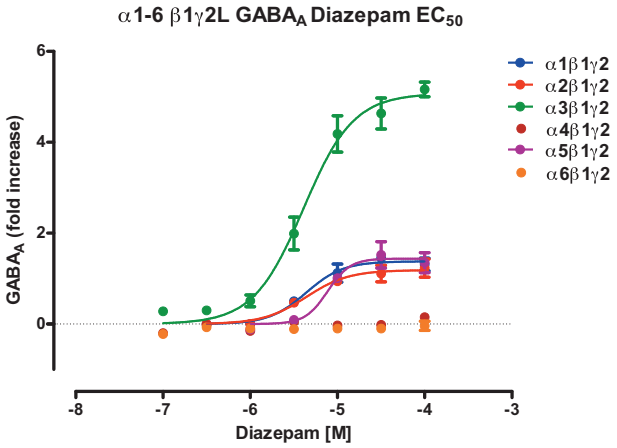


Figure 6. Comparison of fold-increase in activity of $\alpha_1$-$\alpha_6$ ($\beta_1\gamma_2$ containing) GABA$_A$ subtypes in the presence of increasing concentrations of Diazepam.

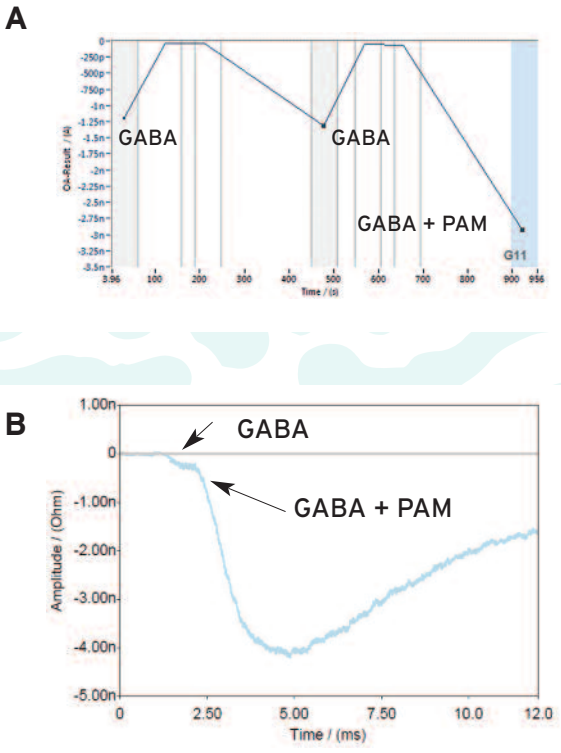


Figure 5. A) Time-course showing effect of PAM on GABA$_A$ currents. B) Example current trace showing $EC_{10}$ GABA followed by GABA plus PAM.

The positive allosteric modulator Zolpidem also displayed a selectivity profile as expected (Figure 7). At 0.1 µM, Zolpidem showed greatest fold-increase in efficacy on the $\alpha_1$ ($\beta_3\gamma_2$) containing GABA$_A$ subtype compared to the $\alpha_2$ and $\alpha_3$ ($\beta_3\gamma_2$) containing subtypes. At this concentration Zolpidem did not show any effect on $\alpha_4$, $\alpha_5$ or $\alpha_6$ ($\beta_3\gamma_2$) subtypes.

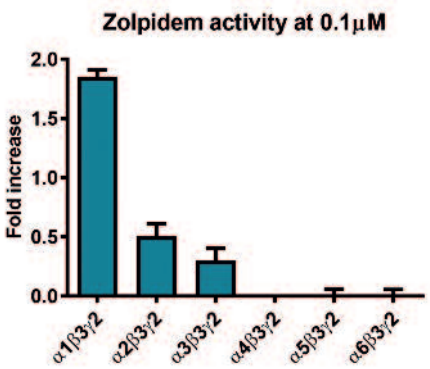


Figure 7. Comparison of Zolpidem efficacy against $\alpha_1$-$\alpha_6$ ($\beta_3\gamma_2$) GABA$_A$ subtypes.

# $GABA_A$ Receptors Application Note

**Multiple assays on a single plate**

The 384-well format offered by the SyncroPatch has enabled development of single plate assays to assess multiple $GABA_A$ subtypes simultaneously. This format allows alpha or beta subunit selectivity to be assessed as well as custom subtype panels, with up to 6 cell lines being assessed per plate (Figure 8).

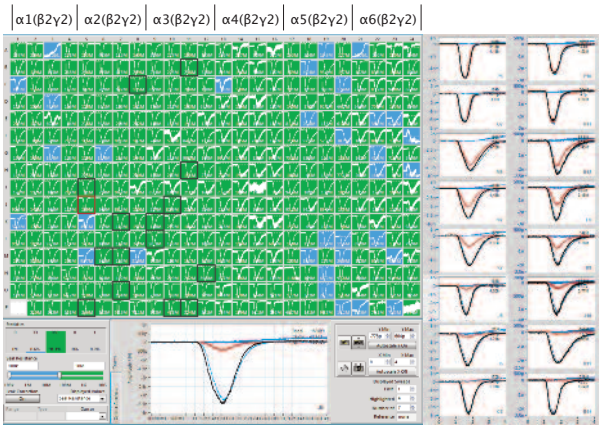


Figure 8. SyncroPatch assessment of six $GABA_A$ subtypes on a single plate.

Using this format in agonist mode we confirmed literature reports showing increased potency of GABA against $\alpha_6$ containing cell lines (Figure 9).

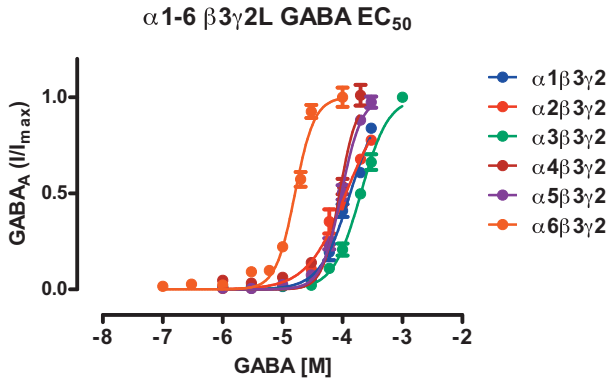


Figure 9. GABA $EC_{50}$ comparison across $\alpha_1$-$\alpha_6$ ($\beta_3\gamma_2$) containing $GABA_A$ subtypes using a single-plate SyncroPatch assay.

**Summary**


$GABA_A$ receptors have been shown to be important drug targets for the treatment of a number or neuronal disorders. Here we have developed and validated an extensive panel of high quality $GABA_A$ receptor cell lines and electrophysiology assays suitable for screening and assessment of small molecules with the ability to modulate the function of these receptors. These high quality cell lines, showing >90% success rate, in combination with the flexibility and throughput offered by the Syncropatch 384PE presents opportunities to rapidly screen and profile large compound sets against a broad panel of receptor subtypes which will undoubtedly lead to a broader understanding of compound profiles.


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