Application Note

Stability of the Wallac LANCE™ Eu-chelates

INTRODUCTION

LANCE™ is a homogenous technology based on time-resolved fluorescence detection. The LANCE TR-FRET assay principle is the energy transfer, which occurs in the proximity of highly fluorescent Eu-chelate and acceptor label, APC; but LANCE assays can also be based on highly fluorescent Eu-chelate and the TR-FQA principle. W1024 Eu-chelate is normally preferred in LANCE assays because it is easy to label due to its small size and biocompatibility. W1024 also has low background at the APC emission wavelength, 665 nm. The other commonly-used LANCE chelate is W8044, which is less sensitive to external interference and has brighter emission than W1024. W8044, however, creates a slightly higher background at 665 nm.

Many screening assays require special buffers for enzyme activity and certain buffer compounds or conditions may have an effect on the stability of europium chelates. Changes in chelate fluorescence can complicate LANCE assays and the ability to control and avoid disadvantageous environmental factors is the key to obtaining reliable screening results. In LANCE TR-FRET assays sample interference effects can be compensated mathematically by a quench correction algorithm, which is available in Wallac ViewLux™, EnVision™ and VICTOR^2™ instruments (TR-FRET models), see reference 1. This paper discusses the effects of pH, EDTA and the most common metal ions on W1024 and W8044 LANCE chelates in common assay concentrations. The information can be applied in both LANCE TR-FRET and TR-FQA assays.

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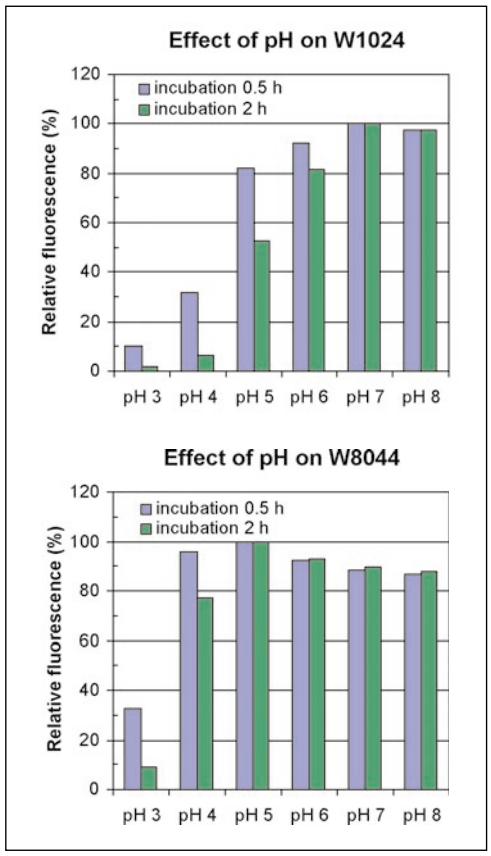
EFFECT OF pH

Biomolecular assays are normally performed in nearly neutral pH where the fluorescence and energy transfer properties of W1024 and W8044 are stabile. At low pH the exchange reactions of the ligand become more rapid and the stability of the chelate can be decreased depending on other compounds present in the assay. The effect of a competing chelating agent on chelate stability may be increased at low pH conditions. Low pH can also cause changes, which might have effect on the energy transfer from ligand to europium.

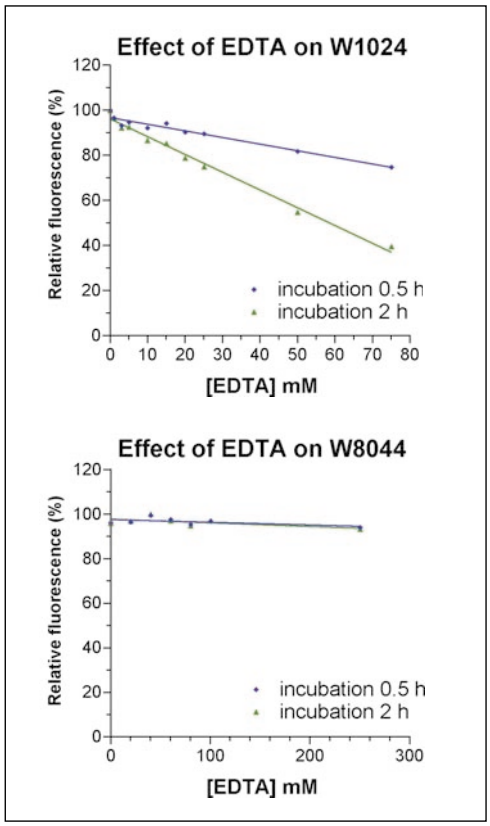


Figures 1 and 2. Relative fluorescence signals of W1024 and W8044 chelates under different pH conditions. Effect of pH was studied by preparing similar dilutions of the chelate in buffer solutions with different pH. Buffers were 50 mM glysine-HCl (pH 3), 50 mM succinic acid (pH 4-6), 50 mM Pipes (pH 7), 50 mM Tris-HCl (pH 8).

The usable pH range of LANCE W1024 continues down to pH 5 but below pH 5 it is strongly quenched. LANCE W8044 chelate has better pH resistance and it remains stabile until pH 4. The use of phosphate buffer at low pH should be avoided because phosphates reduce the usable pH range of the chelates.

EFFECT OF EDTA

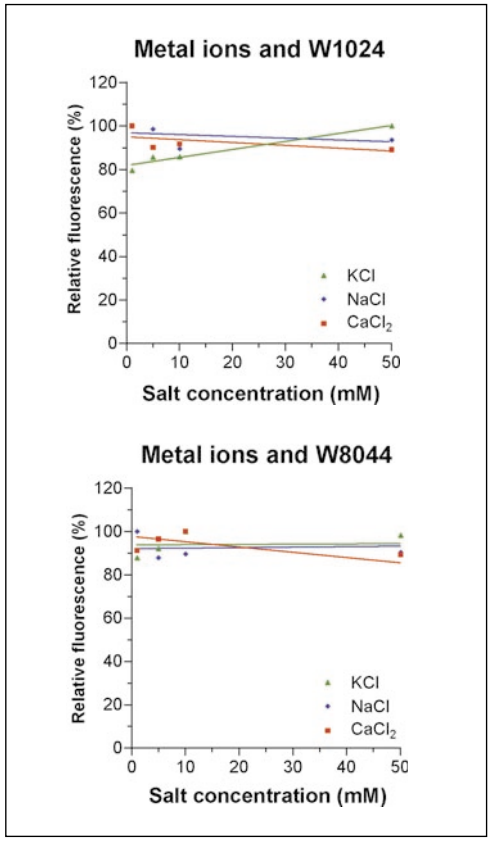
EDTA is a common chelator, which is used to stop enzyme reactions and to bind disturbing metal ions in screening assays. However, it is also possible that EDTA can release Eu^{3+} from its chelate especially during prolonged incubations. Under neutral conditions LANCE chelates resist EDTA very well. W1024 can be used in EDTA concentrations up to 20 mM and W8044 can be used in EDTA concentrations at least up to 250 mM without significant changes in the stability. Low pH decreases the EDTA stability of the chelates.



Figures 3 and 4. Relative fluorescence signals of W1024 and W8044 chelates in the presence of EDTA at pH 7,8.

EFFECTS OF METAL IONS

Metal ions are needed for certain types of enzyme activity, but they can also interact with the chelate and quench the fluorescence. The effect of common metal ions on chelate fluorescence is shown in figures 5–7. Regular physiological ions, such as K^+, Na^+, $Mg^{2+}$and Ca^{2+}, do not have any effect on LANCE chelate fluorescence. Other ions, such as Mn^{2+} and heavy metal cations Cr^+, Co^{2+}, $Fe^{2+/3+}$ and Cu^{2+} quench negatively charged chelates by ion-pair mechanism, if present in assay as free cations. This quenching effect can, however, be prevented with EDTA addition, see figures 8 and 9. When EDTA is used to prevent metal ion quenching of W1024 unnecessary high EDTA concentrations should be avoided because chelate stability can be decreased. Normally the concentration of EDTA should equal the concentration of the disturbing free metal ion.



Figures 5 and 6. Influence of some common physiological ions on W1024 and W8044 fluorescence after 30 minutes incubation.

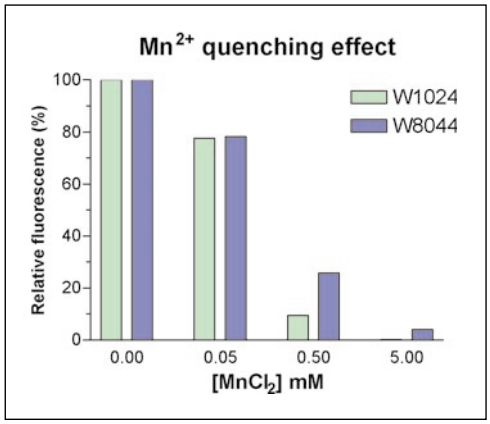
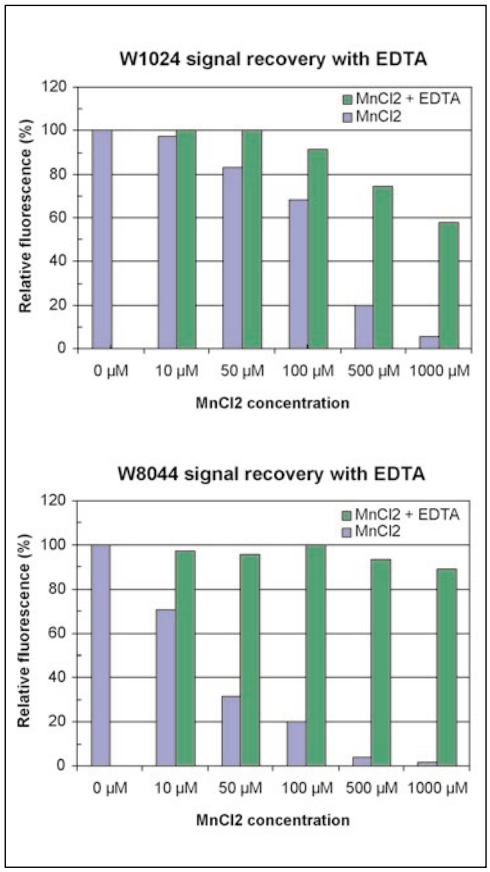


Figure 7."Mn^{2+} quenching effect after 5 minutes incubation.



Figures 8 and 9. Recovery of W1024 and W8044 signal with EDTA addition. 20-fold EDTA excess compared to $MnCl_2$ concentration was added to solutions and fluorescence signal was measured after 30 minutes incubation.

CONCLUSIONS

- W1024 and W8044 chelates are both very stabile under normal screening conditions.
- pH ranges 5-11 for W1024 and 4-11 for W8044 are usable in common buffer solutions. At low pH the exchange reactions of the ligand become more rapid and other compounds present may then have increased effect on chelate stability.
- Under neutral conditions W1024 remains stabile in EDTA concentrations up to 20 mM and W8044 remains stabile in EDTA concentrations at least up to 250 mM. Low pH decreases stability against EDTA.
- Regular physiological ions do not have any effect on LANCE chelate fluorescence. In the presence of disturbing metal ions the quenching of the chelate can be prevented with EDTA addition.
- W1024 provides better S/N-ratio in TR-FRET-assays and it is easy to label.
- W8044 is less sensitive to external interference, and it is recommended under extreme conditions. W8044 also gives brighter signal than W1024.

REFERENCES

1. Application note: Quench Correction for LANCE™ Time-Resolved Fluorescence Resonance Energy Transfer, http://www.lifesciences.perkinelmer.com/library/appnotes.asp

2. Park Y-W., Critical evaluation of homogenous proximity assays. High-Throughput Screening, *IBC's 8th Ann Int Conf*, Berkeley CA, March 1-3 (1999).

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